CONTINUATION APPLICATION

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ON

# GENETIC TEST TO DETERMINE NON-RESPONSIVENESS TO STATIN DRUG TREATMENT

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# GENETIC TEST TO DETERMINE NON-RESPONSIVENESS TO STATIN DRUG TREATMENT

## BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

### 1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular, it relates to the field of genetic testing methods and diagnostic kits.

### 2. DISCUSSION OF THE RELATED ART

Statin drugs-- the most potent lipid-lowering agents currently available-- are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. They include lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin. All these statin drugs share a common mechanism of action and have similar toxicity profiles. (E. von Kreutz and G. Schluter, *Preclinical safety evaluation of cerivastatin, a novel HMG-CoA reductase inhibitor*, Am. J. Cardiol. 82(4B):11J-17J [1998]; A.G. Ollson [1998]).

The statin drugs are effective in reducing the primary and secondary risk of coronary artery disease and coronary events, such as heart attack, in middle-aged and older men and women (under 76 years), in both diabetic and non-diabetic patients, and are often prescribed for patients with hyperlipidemia. (A.G. Ollson, *Addressing the challenge*, Eur. Heart J. Suppl. M:M29-35 [1998]; M. Kornitzer, *Primary and secondary prevention of coronary artery disease: a follow-up on clinical controlled trials*, Curr. Opin. Lipidol. 9(6):557-64 [1998]; M. Farnier and J. Davignon, *Current and future treatment of hyperlipidemia: the role of statins*, Am. J. Cardiol. 82(4B):3J-10J [1998]). Statins used in secondary prevention of coronary artery or heart disease significantly reduce the risk of stroke, total mortality and morbidity and attacks of myocardial ischemia; the use of statins is also associated with improvements in endothelial and fibrinolytic functions and decreased platelet thrombus formation. (M. Kornitzer [1998]; M. Farnier and J. Davignon, *Current and future treatment of hyperlipidemia: the role of statins*, Am. J. Cardiol. 82(4B):3J-10J [1998]).

The use of statin drugs has recently decreased the need for surgical coronary revascularization, known as coronary artery bypass graft (CABG). (B.M. Rifkind, *Clinical trials of reducing low-density lipoprotein concentrations*, Endocrinol. Metab. Clin. North Am. 27(3):585-95, viii-ix [1998]). But CABG is still a common surgical intervention for patients who develop atherosclerotic occlusion in coronary arteries. Approximately 12,000-14,000 CABG procedures are performed annually. (G.F. Neitzel *et al.*, *Atherosclerosis in Aortocoronary Bypass Grafts*, Atherosclerosis 6(6):594-600 [1986]). The

patient's own saphenous vein, or brachial or mammary artery, is used to bypass the affected coronary artery. The majority of CABG patients experience good long-term results, but 30-40% require a second CABG within 10-12 years after surgery, and continuing atherosclerosis in the graft is an important factor in late graft failure. (L. Campeau *et al.*, *The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in saphenous-vein coronary-artery bypass grafts*, N. Eng. J. Med. 336(3):153-62 [1997]).

Atherosclerosis in bypass grafts is associated with elevated serum levels of very low density lipoproteins (VLDL), low density lipoprotein cholesterol (LDL-C), and triglycerides, and low levels of high density lipoprotein cholesterol (HDL-C). (J.T. Lie *et al.*, *Aortocoronary bypass saphenous vein atherosclerosis: Anatomic study of 99 vein grafts from normal and hyperlipoproteinemic patients up to 75 months postoperatively*, Am. J. Cardiol. 40:906 [1977]; L. Campeau *et al.*, *The relation of risk factors to the development of atherosclerosis in saphenous vein bypass grafts and the progression of disease in the native circulation*, N. Eng. J. Med. 311(21):1329-32 [1984]). It is standard for CABG patients to be prescribed statin drugs to lower their serum LDL-C.

Lipid lowering therapy has been demonstrated to delay the progression of atherosclerosis in coronary arteries. (E.g., G. Brown *et al.*, *Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B*, N. Engl. J. Med. 323:1289-98 [1990]; J.P. Kane *et al.*, *Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens*, JAMA 264:3007-12 [1990]; Jukema *et al.*, 1995). Prior to the Post-CABG Trial, few data were available to determine the efficacy of LDL-lowering therapy to delay the obstruction of saphenous-vein grafts. (D.H. Blankenhorn *et al.*, *Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts*, JAMA 257:3233-40 [1987]). Furthermore, thrombosis had also been observed to contribute to graft obstruction (G.F. Neitzel *et al.*, *Atherosclerosis in aortocoronary bypass grafts: morphologic study and risk factor analysis 6 to 12 years after surgery*, Arteriosclerosis 6:594-600 [1986]). Low-dose anticoagulation therapy prevented emboli after major surgery (A.G.G. Turpie *et al.*, *Randomised comparison of two intensities of oral anticoagulant therapy after tissue heart valve replacement*, Lancet 1:1242-45 [1988]; L. Poller *et al.*, *Fixed minidose warfarin: a new approach to prophylaxis against venous thrombosis after major surgery*, Br. Med. J. 295:1309-12 [1987]), and this implied that low-dose anticoagulation treatment would also be able to delay graft obstruction.

Statin drug treatment beneficially affects the long-term outcome for most CABG patients. In a large clinical study, the Post-CABG Trial, CABG patients received statin drug treatment to lower serum LDL-C; in comparing patients who had received aggressive lovastatin treatment (LDL-C lowered to 93-97 mg/dl) to those who had only received moderate lovastatin treatment (LDL-C lowered to 132-136 mg/dl), the percentages of patients with atherosclerotic worsening of grafts within 4 years were 39% and 51%, respectively,. (L. Campeau *et al.* [1997]). The number of patients in the aggressive lovastatin-treatment group who required a second CABG procedure was 29% lower than the number in the moderate-treatment

group.

In addition to serum lipid concentrations, there are other risk factors, that may have a genetic basis, and that may independently affect atherosclerotic coronary artery disease and occlusion of bypass grafts or that interact with statin treatment to lower serum lipids, which can affect atherosclerotic stenosis. Several laboratories have observed a link between variant alleles of the lipoprotein lipase gene (*LPL*) and the occurrence and/or progression of atherosclerosis. The involvement of *LPL* in coronary artery disease was suspected, since rare homozygotes for defects in this gene have type I hyperlipoproteinemia (OMIM 238600) and premature coronary artery disease. (P. Benlian *et al.*, *Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene*, N. Engl. J. Med. 335:848-54 [1996]).

Lipoprotein lipase (LPL; E.C. 3.1.1.34), also known as triacylglycerol acylhydrolase, is a heparin-releasable glycoprotein enzyme bound to glycosaminoglycan components of macrophages and to the luminal surface of capillary epithelial cells in a variety of human tissues, including heart, skeletal muscle, adipose, lung, and brain. (K.L. Wion *et al.*, *Human lipoprotein lipase complementary DNA sequence*, Science 235:1638 [1987]; C. Heizmann *et al.*, *DNA polymorphism haplotypes of the human lipoprotein lipase gene: possible association with high density lipoprotein levels*, Hum. Genet. 86:578-84 [1991]). Lipoprotein lipase is active as a dimer of identical subunits, each approximately 62,500 D in unglycosylated form. (M.R. Taskinen *et al.*, *Enzymes involved in triglyceride hydrolysis*. In: James Shepard (Ed.), *Bailliere's Clinical Endocrinology and Metabolism*, Vol. 1, No.3, Bailliere Tindall, London, pp.639-66 [1987]).

Lipoprotein lipase is the rate-limiting enzyme for the hydrolysis and removal of triglyceride-rich lipoproteins, such as chylomicrons, VLDL, and LDL-C from the blood stream. (Jukema *et al.*, *The $Asp_9$ Asn Mutation in the Lipoprotein Lipase Gene Is Associated With Increased Progression of Coronary Atherosclerosis*, Circulation 94(8):1913-18 [1996]). The enzymatic action of LPL results in the generation of mono- and diglycerides and free fatty acids that can be used as fuel for energy or reesterified for storage in peripheral adipose tissue.

The gene sequence of human *LPL* is known, including the 3' region through exon 10 and the 3' untranslated region (3'-UTR). (K.L. Wion *et al.*, *Human lipoprotein lipase complementary DNA sequence*, Science 235:1638-41 [1987]; T.G. Kirchgessner *et al.*, *The sequence of cDNA encoding lipoprotein lipase*, J. Biol. Chem. 262(18):8463-66 [1987]; K. Oka *et al.*, *Structure and polymorphic map of human lipoprotein lipase gene*, Biochim. Biophys. Acta 1049:21-26 [1990]; D. A. Nickerson *et al.*, *DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene*, Nat. Genet. 19:233-40 [1998]). Nickerson *et al.* sequenced the region of the *LPL* gene spanning exons 4-9 (containing the major catalytic portion of the enzyme) of 71 individuals taken from 3 different populations and observed 88 different DNA variants or polymorphisms, with 78 of these present at an allele frequency greater than 1% (D.A. Nickerson *et al.*, [1998]).

Two *LPL* polymorphisms are known to affect LPL activity. The D9N mutation in exon 2 has been

associated with increased triglyceride levels and with the occurrence of coronary atherosclerosis, attenuating the ability of pravastatin to lower LDL-C. (J. Jukema *et al.* [1996]). The N291S mutation in exon 6 has been associated with reduced HDL-C levels. (P. Reymer *et al.*, *A lipoprotein lipase mutation [asn291ser] is associated with reduced HDL cholesterol levels in premature atherosclerosis*, Nat. Gen. 10:28-34 [1995]; H.H. Wittrup *et al.*, *A common substitution [asn291ser] in lipoprotein lipase is associated with increased risk of ischemic heart disease*, J. Clin. Inves. 99:1606-13 [1997]). The N291S mutation is also linked with increased coronary stenosis (narrowing of arterial lumen) seen on angiography in women with verified ischemic heart disease compared to controls. (H.H. Wittrup *et al.* [1997]).

Two other *LPL* polymorphisms have demonstrated association with the development of atherosclerosis, although their functional significance is unknown. The first is the *PvuII* polymorphism in intron 6, which is linked with the number of coronary blood vessels with greater than 50% obstruction. (X. Wang *et al.*, *Common DNA polymorphisms at the lipoprotein lipase gene: association with severity of coronary artery disease and diabetes*, Circulation 93:1339-45 [1996]). The second is the *Hind*III polymorphism in intron 8, associated with the angiographic severity of coronary artery disease. (R. Mattu *et al.*, *DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population*, Arterio. Thromb. 14:1090-97 [1994]; R. Peacock *et al.*, *Associations between lipoprotein lipase, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden*, Atherosclerosis 97:171-85 [1992]).

Progress in pharmacogenetics has shown that human genetic variation underlies different individual responses to drug treatment within a population. (Reviewed in G. Alvan, *Genetic polymorphisms in drug metabolism*, J. Int. Med. 231:571-73 [1992]; P.W. Kleyn and E.S. Vesell, *Genetic variation as a guide to drug development*, Science 281:1820-22 [1998]). For example, alleles of the *NAT*1 and *NAT*2 genes (N-Acetyltransferases) create a "slow acetylator" phenotype in 40-60% of Caucasians, resulting in a slow clearance and associated toxicity of many drugs including isoniazid and procainamide (K.P. Vatsis *et al.*, *Diverse point mutations in the human gene for polymorphic N-acetyltransferase*, Proc. Natl. Acad. Sci. USA 88(14):6333-37 [1991]). A defect in *CYP*2D6 (a member of the cytochrome P450 family) leads to the "poor metabolizer" phenotype in 5-10% of Caucasians, affecting the metabolism of many drugs including some beta-blockers and antiarrhythmics. (Reviewed in A.K. Daly *et al.*, *Metabolic polymorphisms*, Pharmac. Ther. 57:129-60 [1993]). Some genetic variation can be associated with the accumulation of toxic products, for example treatment of TPMT-deficient (thiopurine methyltransferase) patients with 6-mercaptopurine or azathioprine can lead to a potentially fatal hematopoietic toxicity due to higher than normal levels of thioguanine nucleotides. (R. Weinshilboum, *Methyltransferase pharmacogenetics*, Pharmac. Ther. 43:77-90 [1989]; E.S. Vesell, *Therapeutic lessons from pharmacogenetics*, Ann. Intern. Med. 126:653-55 [1997]).

The presence of multiple genetic and environmental factors capable of creating such large variations in how drugs operate in the patient argues that individualization of the choice of drug and dosage

is required for optimal treatment of disease, including atherosclerotic coronary artery disease. Jukema *et al.* (1996) reported that the HMG-CoA reductase inhibitor pravastatin did not lower the LDL-cholesterol level in subjects with the *LPL* N9 polymorphism to the same extent as in those with the *LPL* D9 polymorphism. In addition, J. A. Kuivenhoven *et al.* (1998) observed that pravastatin slowed the progression of atherosclerosis in subjects with the *CETP* B1B1 genotype, but not in those with the *CETP* B2B2 genotype. (J.A. Kuivenhoven *et al.*, *The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis*, N. Engl. J. Med. 338:86-93 [1998]). These reports suggest that there are interactions between statin drugs and some genetic determinants of atherosclerosis.

There has been a definite need for a reliable predictive test for determining which patients suffering from coronary artery disease, or which CABG patients, will likely not respond positively to statin drug treatment with respect to stenosis of a coronary artery or bypass graft. Such a genetic testing method can provide useful information so that patients can be given more individually suited alternative treatments to prevent further injury.

This and other benefits of the present invention are described herein.

## SUMMARY OF THE INVENTION

The present invention relates to a method of detecting a genetic predisposition in a human subject for non-responsiveness to statin drug treatment for coronary artery disease. This genetic testing method involves analyzing amplification products of the nucleic acids in a human tissue sample that includes a non-coding or untranslated region within the 3' end of the human *LPL* gene. Homozygosity for a variant allele in a non-coding or untranslated region within the 3' end of the human *LPL* gene indicates a genetic predisposition for non-responsiveness to treatment with statin-class drugs, such as lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin, which are typically prescribed to treat atherosclerotic stenosis in subjects with coronary artery disease, or to prevent graft worsening (stenosis) in CABG patients.

The present invention also relates to oligonucleotide primer sequences, primer sets, and genetic testing kits for practicing the method.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows graft worsening in subjects related to different *LPL* variant alleles in the *LPL* gene.

Figure 1(a) shows the location of some variant alleles in the *LPL* gene. Vertical bars represent exons. Figure 1(b) shows the percentage of subjects with graft worsening. Each pair of vertical bars represents two genotype groups for each marker as defined in the box at the base of the bar. The number of subjects in each genotype group (N) is given below each bar. Figure 1(c) represents the odds ratios and 95% confidence limits for graft worsening for each polymorphism.

Figure 2 shows graft worsening in subjects by *Hind*III genotype and drug treatment groups. The

total number of subjects in each group is given on each vertical bar.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to a method of detecting a genetic predisposition in a human subject for non-responsiveness to statin drug treatment for coronary artery disease or high blood pressure. This genetic testing method involves analyzing amplification products of the nucleic acids in a human tissue sample for homozygosity with respect to a variant allele in a non-coding or untranslated region of the 3' end of the human *LPL* gene. The present invention does not rely on and is not committed to any particular mechanism by which a variant allele or *LPL* polymorphism in a non-coding or untranslated region of the 3' end of the human *LPL* gene produces a phenotype of non-responsiveness to statin drug treatment.

The LPL gene is located on the short arm of human chromosome 8, at 8p22. (R.S. Sparkes *et al.*, *Human genes involved lipolysis of plasma lipoproteins: Mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21*, Genomics 1:138-44 [1987]). The gene is near microsatellite marker D8S1715 and flanked by microsatellites D8S261 and D8S280. Closer flanking sequences of human LPL are defined by GenBank accession numbers M94221 and M94222 (S. Wood *et al.*, *Support for founder effect for two lipoprotein lipase [LPL] gene mutations in French Canadians by analysis of GT microsatellites flanking the LPL gene*, unpublished [1992]). The gene spans about 30 kb and contains 10 exons encoding a 475 amino acid protein including a 27 amino acid secretory signal peptide. (S. Deeb and R. Peng, *Structure of the human lipoprotein lipase gene, Biochemistry 28(10):4131-35 [1989]; T.G. Kirchgessner et al., Organization of the human lipoprotein lipase gene and evolution of the lipase gene family*, Proc. Natl. Acad. Sci. USA 86:9647-51 [1989]).

The 3' end of the human lipoprotein lipase gene, for purposes of the present invention, includes nucleotide positions 4801 through 9734 of the Nickerson reference sequence extending from intron 6 into intron 9. (GenBank accession No. AF050163). (D. A. Nickerson *et al.*, *DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene*, Nat. Genet. 19:233-40 [1998]). The complete Nickerson reference sequence is the following:

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   1 TGTAACACAA AATTAAAATA AGTAGAATTA GTTTTCAGTA TTTCCTATAT TTGGAAAACA
  61 ATATTTATAT TCATTTTGTT TCTTTTAGTT TTATTTTTGG CAGAACTGTA AGCACCTTCA
 121 TTTTCTTTTT CTTCCAAAGG AGGAGTTTAA CTACCCTCTG GACAATGTCC ATCTCTTGGG
 181 ATACAGCCTT GGAGCCCATG CTGCTGGCAT TGCAGGAAGT CTGACCAATA AGAAAGTCAA
 241 CAGAATTACT GGTAAGAAAG CAATTTCGTT GGTCTTATCA TAAGAGGTGA AAAGACTGTC
 301 ATTCTGAGAG AGAATCAGAA CAAATTTTGT TAAATACCCA CATGTGTGGT GTTCTTCCCG
 361 GAGACATGAC CAGCACTTGA TTATCTCATT GTAGGGCTCT TTATTAGGGA TAAGAAAAAA
 421 CACAGACGCT CTCACTGGCT TACTATCCAC TGGCAATAGC ACAGAAATAA AGCATAATTA
 481 CACACAATGC CTGCAGATTT CTCTGGGAAG CCTGTTTCCT CCCACTCTCA GCTCTGTGTT
 541 TTAGTAGTGT AAATGCACAT CAGTACTAGG AGAAAAGAAG AAGGACCAAT TCCAGAGGCC
 601 ACTTCGAAAG AAGACCGTCA TCTAGGCAAA GGTGTGGCAT ACACACAGAG AGAAAGAACC
 661 CACCACTGTT TATACATCTT CTCGACATAT TCAGAAATAA TCTACAAAAG GAAATCCAGC
 721 CATCCTGAGT GGAAATTGCT GCATAAGGCT AGTTTAAGAG ACTCAAATTC ATTTTAGAAG
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781 GAGCCAAGCC TCCTTTTATG TCTCTCTAAG TAAAGATACC ATGACTGTAG AATAGGAGCT
841 AATAAGAATC TAAATAGCTG CCAGTGCATT CAAATGATGA GCAGTGACAT GCGAATGTCA
901 TACGAATGGA AATTTACAAA TCTGTGTTCC TGCTTTTTTC CCTTTTAAGG CCTCGATCCA
961 GCTGGACCTA ACTTTGAGTA TGCAGAAGCC CCGAGTCGTC TTTCTCCTGA TGATGCAGAT
1021 TTTGTAGACG TCTTACACAC ATTCACCAGA GGGTCCCCTG GTCGAAGCAT TGGAATCCAG
1081 AAACCAGTTG GGCATGTTGA CATTTACCCG AATGGAGGTA CTTTTCAGCC AGGATGTAAC
1141 ATTGGAGAAG CTATCCGCGT GATTGCAGAG AGAGGACTTG GAGGTAAATA TTATTTAGAA
1201 GCGAATTAAA TGTGACTCTT ATCCTTAACC CTTATTGACC CAATGTCCTA CTCAGTAGCT
1261 TCAAAGTATG TAGTTTTCAT ATACACATTT GGCCAAATTA TGTTTCTGAA GAATTCTGCA
1321 ATGTTCAGCA TGACCACCTT AGAGCCAGGC AGACAGCCAT TTTATCTTTT ATTTACTATA
1381 CTGTAGGCTA CACTGAGCAG TGCACTTACA GTAGCAAGAG AAAAAGGTGG GATTTTAGAC
1441 AGGAAGACTC CACTGACCTC AATAATGGCA TCATAAAATG CTATCTGGCC ACATGTTGTC
1501 ATACCTTGAA TGTAGCTGCA AAGCCAATGG AAAGATTTTA GATGTTACTG GAACAGAAGA
1561 TGTTAATTAG CATAAATCTT CCAAAATGTT CAGAACATAA TGTTAGCTTA ATGTTTTACT
1621 TTAATAATGT TAGCTTGTGT TAAATTTATG ATTTTTGTTT GTTTGTTTTT TGAGATAGAG
1681 TCTTATTCTA TTGCCCAAGC TGGGGTGCAG TCACACAATC ACAGGGACTT GCAATGTTGC
1741 CCAGGCTGGT CTCAAACTCC TGGCCTCAAG TGATCCTCCT GCCTCAGCCT CCCAAAGTTC
1801 TGGGATTGCA GCTGTGAGCC ACCACGCCCA GTTTACGATT TATTTTTAAG AGCCCCTTGC
1861 ATACTTTATA GACATTGGGA CCTACCTAGG ATATTCTCGT TATTTTTGTG CACGTAATAG
1921 AACTTAGAGC ATATTGTTAC TATTTTCGAT TGTCCTAAAA ACTTACAAGG AATTCATTCT
1981 TATGGCATTG CTGATTATTT CTATGTTCAT TTGATATAAA AGAGTGTTAG TAGGGGCAGA
2041 ACCCTCAATT GTACATAATA TCAATGATAA AATACAATTC ATTTAACAAT TACCCTCTTA
2101 AGATGTGGTT TCTAGAAATA CAAATTGTCC CTAACTTACA GTTTTCCAAC TTTACAATTG
2161 GGCTGTAACA CCATTTTAAG TTGAGAAGCA CGTGATGGTT TGACTTAAAA CTTTTTGACA
2221 TTATGATGGG TTTTGGGGGT ATTAAGTGCA TTTTGACTTA CAGTATTTTT GACTTATGAA
2281 GAATTTATTG TAAGGCAAGG GGCAGGTATA TGTTTCTAGA AGCACCTAGA AGTGTTAGAC
2341 ACTTTCAATG TAAGAGAAGG ATGAGATAAA CAAGGAAATC ACACCTCCAC CTTGGAGGCT
2401 TATTACAGCT TCATAAACAT ACTCATAAAT ATAAGAAGCA CAAAAGTCAA AAATTCCCTG
2461 TGAACTTGCA ACTTTCACTC TCTTGAAGGT GGGTGGGCCG CTACCACCAA GAATATCTCC
2521 TGAAATAGGG CCTACAATCA TAAATGCACA GGACTATATC CTTGGGTGAT TCTACTCTAA
2581 CACCACATCT CACCTATTTT AGACATGCCA AATGAAACAC TCTTTGTGAA TTTCTGCCGA
2641 GATACAATCT TGGTGTCTCT TTTTTACCCA GATGTGGACC AGCTAGTGAA GTGCTCCCAC
2701 GAGCGCTCCA TTCATCTCTT CATCGACTCT CTGTTGAATG AAGAAAATCC AAGTAAGGCC
2761 TACAGGTGCA GTTCCAAGGA AGCCTTTGAG AAAGGGCTCT GCTTGAGTTG TAGAAAGAAC
2821 CGCTGCAACA ATCTGGGCTA TGAGATCAAT AAAGTCAGAG CCAAAAGAAG CAGCAAAATG
2881 TACCTGAAGA CTCGTTCTCA GATGCCCTAC AAAGGTAGGC TGGAGACTGT TGTAAATAAG
2941 GAAACCAAGG AGTCCTATTT CATCATGCTC ACTGCATCAC ATGTACTGAT TCTGTCCATT
3001 GGAACAGAGA TGATGACTGG TGTTACTAAA CCCTGAGCCC TGGTGTTTCT GTTGATAGGG
3061 GGTTGCATTG ATCCATTTGT CTGAGGCTTC TAATTCCCAT TGTCAGCAAG GTCCCAGTGC
3121 TCAGTGTGGG ATTTGCAGCC TTGCTCGCTG CCCTCCCCTG TAAATGTGGC CATTAGCATG
3181 GGCTAGGCTA TCAGCACAGA GCTCAGAGCT CATTTGGAAC CATCCACCTC GGGTCAACAA
3241 ACTATAACCC TTGTGCCAAA TCCAGCCTAC TTCCTGCTTT TGTAAATAGT TTTTTTAAAA
3301 CTTTTAAGTT CAGGGGTACG TATGTAGGTT TGCTAAAAAG GTAAACTTGT GACATGGGAG
3361 TTTGTTGTCC AGAATATTCC ATCACCCAGG TATTAAGCTT AGTACCCATT AGTTACTTTT
3421 CCTGAAGCTC TCCCTCCTCC CACCCTCTGG GAGGCCCCAG TGTCTGTTGT TCCCCTCTAT
3481 GTGCTCATGC AAAGTTTTAT TAGGACACAG CCACACACAT TCATTACCAT ATTGTCAAAG
3541 GCTGGTTTCA TGCCACCATA ACAGAGTTGA TAGCCCACAG AGCCTAAAAT ATTTACTCCC
3601 TGGCCCTTTA CAGAATGTTC ACAACTTACA TAAAGGCAAG GACCATCTGT CTTATTTATT
3661 TATTTATTTA ATTTGAGATG AAGTCTAGCT TTCTCCTAGG CTGGAGGAGA GGGGCATGAT
3721 CTTGGCTCAC CACAACCTCT GCCTCCCGGG TTCAAATGAT TCCCCTGCCT CAGCCTCCGG
3781 AGTAGCTGGG ATAACAGGCA TGCACCATCA TGCCCAGCTA ATTTTTGTAT TTTTAGTAGA

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3841 GAGGGGGTTT CACCGTGTTG ACCAGGCTGG TCTCGAACTG CTGACCTCAG GTGATCTGCC
3901 CTCCTTGGCC TCATCTGTCT TTTTAAATGC AACTATTCCT GGAAGGCAAG AATATCTCAC
3961 ACCTTCTAAG ATACTGCCAT TTTGCCAGGA GTTTGTTTCA CACTTGAATT TCAAGCTTGG
4021 CCTCTTGTTT AGAGGCAGAC CTAAAGGAAT GGTCGGAAAA TGAGAGAGGA GGTCTTCGGA
4081 TAAATCCGGT GAGAGGGACC AACTTCAGGA AGGGTGGCTT TTGTGGAATC CAGATGGAAA
4141 CCTGAGGGAA GGGATGATAT TAAAGAACAG TGGCCCCAGG TAAAACATAT GGCACCCATG
4201 TGTAAGGTGA TTCTTAGAAT CTGTAGAGGT GTCTTTCGTG GTATAGAGGT TGAGGCACCT
4261 GTGCTTCAAG GAAACCTTAA CTCTTCAAAA TCAGGCAATG CGTATGAGGT AAAGAGAGGA
4321 CTGTGGGACC ATAATCTTGA AGACACAGAC AGGCTTCACT CATCCCTGCC TCCTGCACCA
4381 GTGGGTTCAA GGCTCTGTCA GTGTCCCCTA GGGGCACCTC ACCACTCCCA GCTTCTTCAG
4441 CTCTGGCCTG TCCTGCTGCC TGCAAGGGTT TTGCTTAATT CTCAATTCAA TGTCTCTTCA
4501 TCTTTTAGTA GCTGTGGGGT TTTGTTGTTG TTCTTCTGTT TTTGCTTAGT ATCTGACTAC
4561 TTTTTAATTA TAAAAAGAGA TGTATCTAAA CAAAATAGAG ATTGTTATCA GAAGTTCACA
4621 ACATTTATTA AAAATTTTTT CACCTGGACA AGAGTCTAAA GCAGCATAAA AATATGGTCT
4681 GCTATATTCT AAACCATCAG TCTTAAGAGA TCTGTGTCTC AGCTTAAGAG AAAATACATT
4741 TAATAGACAG TAACACAAAT AAGAAAAAAA TCTGACCAAG GATAGTGGGA TATAGAAGAA
4801 AAAACATTCC AAGAATTATT TTATTTATTT ATTTATTTAT TTATTTATTT ATTTATTTAT
4861 TTTTGAGACA CGGTCTCGCT CAGTTACCCA GGCTGGAGTG CAGCGGCGCA ATCTTAACTC
4921 ACTGCAACCT CTGCTTTCCG GTTCAAGCGA TTCTCCTGCC TCAGCCTCCT GAGTAACTGG
4981 GATTACAGGC ACCCGCCACC ACGCCCAACT AATTTCTGTA TTTTTCTTAG TAGAAACAGG
5041 GTTTCACCAT GTTGGCCAAG CTAGTCTCAA ACTCCTGACC TCAGGTGATT CACCCACCAA
5101 GGCCTCCCAA AGTGCTGGGA TTACAGGCAT GAGCCACCAT GCCTGGCCTC CAAAAACTCT
5161 TTTTTCCTCC ATCATCATGG TTCTATTTTA GTCCTGCTGC CTTTCCTTTT AACCTCTCCC
5221 CAGGCCCATT TGCTCAGGGT TTTTGGTAGA GACCAGAGGA GGGGCAGGGA GGAGATATAG
5281 AAGTTCAACT ACCTGCTTCC AGAGGCTGTC CCTAGTATAG AATACTTTAG GGGCTGGCTT
5341 TACAAGGCAG TCCTTGTGGC CTCACTGATG GCTCAATGAA ATAAGTTCTT TTTTAAAAAA
5401 AATTTTATTT ATTTCCATAG GTTATTGGGG GAACAGGTGG TGTTTGGTTA CATGAGTAAG
5461 TTCTTTAGTA GTGATTTGTG AGATTTTGGT GTGCCCATTA CGGAATGGAA AAATCAACGA
5521 AATAAGTTCT ATGATGCACC TACTAGACAC CTAATCTGCA CTAGATGGTG GGGGAATTAA
5581 GAGCATGGGC ATGATCCTGT GACCGGAAGC CCGCTTACAG TCAGGGTGGA GGACAGACCT
5641 ACTCATGAAA CAAACACAGT GACATATAGT GACACAGAAG CAAATGTCAA ATATGCTTGC
5701 TCCAGATGCT AAGGCACAAG ATGGCCAAGG ATGGCGGAGT TCATGGAGAA AGCATCATGA
5761 GTGTTTTGGC CTTCTGATTT GATCTCCCTA GCACCCCTCA AAGATGGCTA CTTCCTAATG
5821 CTGCTTGGCA ATTCAGACAC ATTTGGGTTT TTCCTATGCA TATAACCACA CTTTTCTGAA
5881 AGGGAGTAGA ATTCAAGGTC TGCATTTTCT AGGTATGAAC ACTGTGCATG ATGAAGTCTT
5941 TCCAAGCCAC ACCAGTGGTT CCATGTGTGT GCACTTCCGG TTTGAGTGCT AGTGAGATAC
6001 TTCTGTGGTT CTGAATTGCC TGACTATTTG GGGTTGTGAT ATTTTCATAA AGATTGATCA
6061 ACATGTTCGA ATTTCCTCCC CAACAGTCTT CCATTACCAA GTAAAGATTC ATTTTTCTGG
6121 GACTGAGAGT GAAACCCATA CCAATCAGGC CTTTGAGATT TCTCTGTATG GCACCGTGGC
6181 CGAGAGTGAG AACATCCCAT TCACTCTGTG AGTAGCACAG GGGGGCGGTC ATCATGGCAC
6241 CAGTCCCTCC CCTGCCATAA CCCTTGGTCT GAGCAGCAGA AGCAGAGAGC GATGCCTAGA
6301 AAACAAGTCT TTAGTTAAAA AAATCAGAAT TTCAAAATTG AGGTCTTTCC TCTATTTGAT
6361 ATTGAGAAAA AAATGCTTCA AATTGGCCAT TTTATTTTCA CTTACTAGTT ATATTTTTTT
6421 ATTTATCATC TTATATCTGT TTATTTCTTT TATAAAGCTG CTGTTAAACA ATATAATTAA
6481 ACTATCTCAA AAGGTTTGAC ATTAAAGAAA ATGAGCAATG GTAACAGGAA ACCACTCTAT
6541 AGATGTACAT ATAATATGTA CAGAAAATAT AAGTAGTAAG AAGTCCATGA CAAAGTGTTA
6601 GCTCTTTTTT TTTTTTTTTT TTTTTTTTTT TTTGAGATGG AGTCTCTCTC CTATTGCCCA
6661 GGCTGGAGTG CAGTGATTCG ATCTCAGCTC ACTGCAACCT CTACCTCCCG AGTTCAAACA
6721 ATTCTTCTGT CTCAGCCTCC CGAGTAGCTG GGGCTGCAGG TGCCCACCAC CATGCCCAGC
6781 TAATTTTTGT ATTTTTAGTA GCGACAGGGT CTCACCATGT TGGCCAAGCT GGTCTTGAAT
6841 TCCTGATCTC AGGTGATCCA CCCGCCTCGG CCTCCCAAAG TGCTGGGATT ACAGGTGTGA
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6901 GCCACCATGC CCAGCCTACC CTTTACTACT AATCAAAGAA ATAAAAGTAA GGCAACTTGA
6961 TACTTTTACA ATTACTAGAT GAACAAATCT TTAAAAATAG CCAGTGCAGA CAAGGTGGTG
7021 AAGCAGAACA TGCGAACCTA CCATGCATCA TTCACGGCTA GAACCCTCCA GGTGCGGAAG
7081 GTAGTATTTT AATAACTTTC CATAGCTACA AAATATTATT ACATAGAAGG GAGTGATTTT
7141 TTTCTAATAT TTATCCTAAA GAAATAGTCA ACAAACATTT TTAAAAAACA TCAATTACAG
7201 TCGTACCTAT ACTAGCATAA ATTAGAAACC CAGTATCCAA CATTGAGGCA GTGGGTAAAT
7261 GAATCGTGGT TTATCAAGTC ATTAAAATCA ATCTAGCCTT TAAAAACTAT AATTGTAGGA
7321 AACCCAGGAA AACATAGTAA AAAATGGAAT ATAAAATCTA AAGAGAATAA AGAATAGAGA
7381 ATCGTATGTG TGCTATGATT GTAGCTAAAT AATGTTCAAG TATCAACACA AATTGAAAAG
7441 GAATACATGA AAATGAAAAT TATATTTCTG AATGATTGAC TTCAGGATTT TCTTTTAGAA
7501 TTGTATTAAA TAGTTCATGT CATTAGGATA AATGCTGGAA TGTGGATATA ATTTAAAATA
7561 TACTAAATGC CATCGACCTT CATTTTGAGT TCTTTGTTGG ACATTTTTGT GCATTTTTAA
7621 AATATCCCCT AAATAATAAA GCTATTTATA TTTGGAGAGG AGAAAAAAAA GTGGGGGGCA
7681 GGGAGAGCTG ATCTCTATAA CTAACCAAAT TTATTGCTTT TTTGTTTAGG CCTGAAGTTT
7741 CCACAAATAA GACATACTCC TTCCTAATTT ACACAGAGGT AGATATTGGA GAACTACTCA
7801 TGTTGAAGCT CAAATGGAAG AGTGATTCAT ACTTTAGCTG GTCAGACTGG TGGAGCAGTC
7861 CCGGCTTCGC CATTCAGAAG ATCAGAGTAA AAGCAGGAGA GACTCAGAAA AAGTAATTAA
7921 ATGTATTTTT CTTCCTTCAC TTTAGACCCC CACCTGATGT CAGGACCTAG GGGCTGTATT
7981 TCAGGGGCCT TCACAATTCA GGGAGAGCTT TAGGAAACCT TGTATTTATT ACTGTATGAT
8041 GTAGATTTTC TTTAGGAGTC TTCTTTTATT TTCTTATTTT TGGGGGGCGG GGGGGGAAGT
8101 GACAGTATTT TTGTATTTCA TGTAAGGAAA ACATAAGCCC TGAATCGCTC ACAGTTATTC
8161 AGTGAGAGCT GGGATTAGAA GTCAGGAATC TCAGCTTCTC ATTTGGCACT GTTTCTTGTA
8221 AGTACAAAAT AGTTAGGGAA CAAACCTCCG AGATGCTACC TGGATAATCA AAGATTCAAA
8281 CCAACCTCTT CAAGAAGGGT GAGATTCCAA GATAATCTCA ACCTGTCTCC GCAGCCCCAC
8341 CCATGTGTAC CCATAAAATG AATTACACAG AGATCGCTAT AGGATTTAAA GCTTTTATAC
8401 TAAATGTGCT GGGATTTTGC AAACTATAGT GTGCTGTTAT TGTTAATTTA AAAAAACTCT
8461 AAGTTAGGAT TGACAAATTA TTTCTCTTTA GTCATTTGCT TGTATCACCA AAGAAGCAAA
8521 CAAACAAACA AAAAAAAAAA GAAAAAGATC TTGGGGATGG AAATGTTATA AAGAATCTTT
8581 TTTACACTAG CAATGTCTAG CTGAAGGCAG ATGCCCTAAT TCCTTAATGC AGATGCTAAG
8641 AGATGGCAGA GTTGATCTTT TATCATCTCT TGGTGAAAGC CCAGTAACAT AAGACTGCTC
8701 TAGGCTGTCT GCATGCCTGT CTATCTAAAT TAACTAGCTT GGTTGCTGAA CACCGGGTTA
8761 GGCTCTCAAA TTACCCTCTG ATTCTGATGT GGCCTGAGTG TGACAGTTAA TTATTGGGAA
8821 TATCAAAACA ATTACCCAGC ATGATCATGT ATTATTTAAA CAGTCCTGAC AGAACTGTAC
8881 CTTTGTGAAC AGTGCTTTTG ATTGTTCTAC ATGGCATATT CACATCCATT TTCTTCCACA
8941 GGGTGATCTT CTGTTCTAGG GAGAAAGTGT CTCATTTGCA GAAAGGAAAG GCACCTGCGG
9001 TATTTGTGAA ATGCCATGAC AAGTCTCTGA ATAAGAAGTC AGGCTGGTGA GCATTCTGGG
9061 CTAAAGCTGA CTGGGCATCC TGAGCTTGCA CCCTAAGGGA GGCAGCTTCA TGCATTCCTC
9121 TTCACCCCAT CACCAGCAGC TTGCCCTGAC TCATGTGATC AAAGCATTCA ATCAGTCTTT
9181 CTTAGTCCTT CTGCATATGT ATCAAATGGG TCTGTTGCTT TATGCAATAC TTCCTCTTTT
9241 TTTCTTTCTC CTCTTGTTTC TCCCAGCCCG GACCTTCAAC CCAGGCACAC ATTTTAGGTT
9301 TTATTTTACT CCTTGAACTA CCCCTGAATC TTCACTTCTC CTTTTTTCTC TACTGCGTCT
9361 CTGCTGACTT TGCAGATGCC ATCTGCAGAG CATGTAACAC AAGTTTAGTA GTTGCCGTTC
9421 TGGCTGTGGG TGCAGCTCTT CCCAGGATGT ATTCAGGGAA GTAAAAAGAT CTCACTGCAT
9481 CACCTGCAGC CACATAGTTC TTGATTCTCC AAGTGCCAGC ATACTCCGGG ACACACAGCC
9541 AACAGGGCTG CCCCAAGCAC CCATCTCAAA ACCCTCAAAG CTGCCAAGCA AACAGAATGA
9601 GAGTTATAGG AAACTGTTCT CTCTTCTATC TCCAAACAAC TCTGTGCCTC TTTCCTACCT
9661 GACCTTTAGG GCTAATCCAT GTGGCAGCTG TTAGCTGCAT CTTTCCAGAG CGTCAGTACT
9721 GAGAGGACAC TAAG (SEQ.ID.NO.:80).

Also for purposes of the present invention, the 3' end of the human lipoprotein lipase gene

includes exon 10 and the 3' untranslated region (3'UTR), at least partially defined by nucleotide positions 1 through 3240 of the reference sequence of Oka *et al.*, (GenBank accession No. X52978 and X53518; K. Oka *et al.*, *Structure and polymorphic map of human lipoprotein lipase gene*, Biochim. Biophys. Acta 1049(1):21-26 [1990], Erratum:[Biochim Biophys Acta 1991 Nov 11;1090(3):357]). In the reference sequence of Oka *et al.*, the first and second polyadenylation signals are at nt. 15-20 and 411-416, respectively (in bold), and two analogous AGTAAA sequences are at nt. 468-473 and 529-534 (in bold). The poly(A) addition site is at nt. 439. The following is the reference sequence of Oka *et al.*:

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   1  GAATTCTCTC TAAAAATAAA ATGATGTATG ATTTGTTGTT GGCATCCCCT TTATTAATTC
  61  ATTAAATTTC TGGATTTGGG TTGTGACCCA GGGTGCATTA ACTTAAAAGA TTCACTAAAG
 121  CAGCACATAG CACTGGGAAC TCTGGCTCCG AAAAACTTTG TTATATATAT CAAGGATGTT
 181  CTGGCTTTAC ATTTTATTTA TTAGCTGTAA ATACATGTGT GGATGTGTAA ATGGAGCTTG
 241  TACATATTGG AAAGGTCATT GTGGCTATCT GCATTTATAA ATGTGTGGTG CTAACTGTAT
 301  GTGTCTTTAT CAGTGATGGT CTCACAGAGC CAACTCACTC TTATGAAATG GGCTTTAACA
 361  AAACAAGAAA GAAACGTACT TAACTGTGTG AAGAAATGGA ATCAGCTTTT AATAAAATTG
 421  ACAACATTTT ATTACCACAC TAAGTCATTA TTTTGTATCA TTTTTAAAGT AAATTTATTC
 481  TTAGGTCAGA TTCACTCAGC ATATTTTGAC TAAGTAACCA CTGTACTTAG TAAACCGAAG
 541  AGCTTCTGAG AATTATAGTG TACCGTATAG ATATTTTTAA CATTTATATT TGTATAAAGC
 601  TAAAGAAAGC CTTACATATC CTTTAAACTG ACTATAGAAG AAAATGATAC AGAATTTTGC
 661  CTGCATAAAG TACACAGGAC TATTCTTGCC TACAATATGC TTTTTCACAA GCAAAATGTT
 721  AGACTAATAT AAGGCATCTT TGGCCATTTT ATAGTGTACA TCATCTCTAT TTCTGAGGCC
 781  TCATTGTTAG CTGTAACGCA AGTAGCATTT GTGCAATAAA ATGAACTATT TGGGATGGGA
 841  GGGTACATTT TTTAGAACTT TGCTTTGGGT TGCCTTGATA ATTAATAGCA TATAGTCCAT
 901  TTATGCAGCT AAGTAGGGAT TGCTTCTTAG TACAGTCAGG AAGAATTTAG CCCAGAAAAC
 961  AATTATTTCA ATGGCCACTG ACCCAAACTT CCAGGCTGAA GAGCAATGGC GTGATCATGG
1021  CTCACTGCAC CTCCACCTCC CAGGCTCAAG TGATTCTCCT GCCTCAGCCT CCCAAGTAGA
1081  TGGTACTACA AGCACACGCC ACTGCACCCA GCTAATTTTT GTATTTTTTG TAGAGATGGG
1141  GGTTTCACCA TGTTGCCCAG GCTGGTCTTA AATTCCTGGC CTCAAGTGTC TGCCCCCCTT
1201  GGCCTCCCAA AGTGCTGGAA TTACAGGCAT GAGCCACCAT GTCCAGCCTT GACCCAAACT
1261  TTTATTGTCA GTTAGCTATT GGGGGCTTCT GGAGTTTGGG TCTCCCCTGA CAGGAGGGGG
1321  CTCCCCAGTT CACACTTGGC CACTGCCCAT CAATTCCTGT TGATATGATC AACAAGATAG
1381  ACAATTGCAA ATGTTGCTGA GGATGTGGAG AAGTGTGAAC CTGTGTAAGT GGCTGATGGG
1441  AATGTAAAAT GGCACAGCCA CTATGGAGAA CAATTTGGTA GTATTTCCAA AGTTAAGCAT
1501  AGAGTTTAAC CCATATGACC CAGCAATTCC ACTCCTAGAT ATATACCCAA GAGAAATGAA
1561  AACACAGATC CACAAAGATT TGCACACACA GGTTCATAGC AGCATTAATC AGATTAGTCC
1621  CAAAGTGGAC AACCCAAATG TCCATGAACT TGTGAAAGAG ATAAGCAAAA TGTGACAAAT
1681  TCACATAATA AAATATTATT CAGAAGTAAA AAGAACAAGC AGCAGATATA TGATACAACA
1741  CGATGCGCCT TGAAAACGTT TAGCCATATG AAAGAAACCA GATGCAAAAT GGAACCATGG
1801  CTTAGGGGAG GAGAACGGCA CAATGGTGTA AAAGTTGCAG AGAGGAACAA AAAGGCTACC
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1861 TGCCTCGCTC CCAGGCCAAG TAACACAGGA GGAAAGAAAA TATCCACATA TGCGAGGGCT
1921 AAAGGAAAGA GGTGTTCTCA AGCTGAAGCA GGAGGTGGGA CTCAACTCTG GAGGTGGGCC
1981 TCACACACTG TACCAAATTG AGGACTAGCT AAAACAGGGA TGGGGGTGAA AGCACCTTTT
2041 CGTAAGACAT GCCCACCATT GTCCCGTTCT CCTCCCTTAA GCCCTTGTCT TGCTCATGTC
2101 AGCAAGCTTA TTGCCATCTA TTCTTCCTAG TTACAGACAT CTGTGGAGCT CTGAGTTTTT
2161 TGCCTAATCA TTATTTTAGA ACCTGGTTCA CTCTCTCTCC CTTCTACACT AGTTCTGTCA
2221 TTATTATTAC TGATTTCAGT ACCTCTGAGG TGATAGATTT TATTTTCCAA TGGCAGCCAC
2281 AACACTACCT CCCATTCTAT ATGTTCCCCT GCAATGTTGC CTTGACATCC CTATTAAGAG
2341 TTGGAATCTA GTCACCCCGC TTTTCTAGTC TCCCCACTCC TTTGAACTTG TGTGGGCCCT
2401 AAGATTGCTT CTACTAGTAG AATAGAACTA AAATGACCCT GGACCAGTGT GGGGTGCAGC
2461 CCTTAACTGG CCTGGCAGCT TCTGCTTTTG GTTCCTTGGG GCACTCACTC TTGGGAAACT
2521 TCCCTTTGGA ACTCAGCATT CATGATGCGG AAGTTGAAGC CACATGAAAA GAGCATATGG
2581 TGGTTCTCTC AGCTCCCAGC CAACAACCAG TCTCGACTGT CAGCCATGTG AGTGAGGCAT
2641 CTTGGACCTC CGGCCAGTTG AGTGTTCAGA AGACTGCAGC TCGAGCTGGC ATCTGGATGC
2701 AACCACATGA GAGACGCTCT GCCCAGCCAA GCCCAGCCAA CTCACAGTAC TATGAGAGAT
2761 ACTAATAACT TGTTGTTGTT GTTGTTGTTG TTGTTTTTAT TATTAAACTT TAAGTTTTAG
2821 CATACACGTG CACAACGTGC AGGTTAGTTA CATATGTATA CCTGGGCCAT GTTGGTGTGC
2881 TGCACCCAGT AACTCGTCAT TTAACATTAG GTATATCTCC AAATGCTATC CCTCCCCCCT
2941 CCCTAAGTTT TTAGGAGTTT GCTTTGCAAC GATAGATAGT TGAAACATCT GGATGATGCA
3001 TCCAGTATTC TGGCTTCTCA CTGCCTTTAC CTCCTCTCTC CCATGGCCTT GTCTTCTAAA
3061 TCTACCTTTA CATAGAAACA TTCAGTCACG TGCTATACTA TATCATGCCA TTACTAATAA
3121 CTCATAAACT CAATTTCAAC TTCTCCCTTC TTTGACTACC ACATGCTATC TTTTTACTTT
3181 AATCAGTCTA GTGCTCTCAG TTCAACAGCT CCTCAACTGC CCCAGGACCT CCAATACATT//

(SEQ. ID.NO.:94).

Also for purposes of the present invention, the 3' end of the human lipoprotein lipase gene includes the intervening nucleotide sequence between the end of the Nickerson reference sequence in intron 9 and the beginning of the reference sequence of Oka *et al.*

A non-coding or untranslated region of the 3' end of the human *LPL* gene includes any non-transcribed or untranslated nucleotide sequence within the 3' end, including all intronic sequences. Included are the part of intron 6 extending from Nickerson reference sequence position nt. 4801 through nt. 6086; intron 7 from nt. 6208 through nt. 7729; intron 8 from nt. 7913 through nt. 8941; and intron 9 from nt. 9047 through nt. 9734. Also included is exon 10 and the 3'UTR.

A variant allele in a non-coding or untranslated region of the 3' end of the human *LPL* gene is a mutation or polymorphism with respect to the Nickerson or Oka *et al.* reference sequences, of any class, such as, but not limited to, a single nucleotide polymorphism (SNP). Included among the sources of variant alleles in a non-coding or untranslated region of the 3' end of the human *LPL* gene are deletion mutations, insertion mutations, inversions, translocations, transitions, tranversions, or repeats.

Examples of homozygous genotypes that indicate a genetic predisposition to non-responsiveness

to statin drug treatment, in accordance with the present method, include, but are not limited to, the *Hind*III 2/2 and $(TTTA)_n$ 4/4 genotypes.

The *Hind*III 2 variant allele, is created by a T to G transition in the single *Hind*III recognition site mapped in intron 8, i.e., AAGCTT to AAGCGT, at position 8393 of the Nickerson reference sequence. (K. Oka *et al.* [1990]; C. Heinzmann *et al.*, *RFLP for the human lipoprotein lipase [LPL] gene: HindIII*, Nuc. Acids Res. 15:6763 [1987]; D. A. Nickerson *et al.* [1998]). For purposes of the present invention, nucleic acids comprising the normal locus of *the Hind*III recognition site in intron 8 of the human *LPL* gene are any nucleic acid sequences that overlap the entire six-basepair region at positions 8389-8394 of the Nickerson reference sequence, whether or not the nucleic acid sequence of a particular human subject at that locus is AAGCTT.

The tetranucleotide $(TTTA)_n$ repeat sequence in intron 6 of the *LPL* gene begins at position 4819 of the Nickerson reference sequence and extending to position 4864. There are five known $(TTTA)_n$ alleles or polymorphisms. Allele 4 yields a 131 bp nucleotide fragment when PCR amplification is done using a primer set comprising reverse primer GZ-15 (5'-CCT GGG TAA CTG AGC GAG ACT GTG TC-3'; SEQ. ID. NO.:33) and forward primer GZ-14 (5'- ATC TGA CCA AGG ATA GTG GGA TAT A-3'; SEQ. ID. NO.:34).

In the $(TTTA)_n$ 4 variant allele, two additional TTTA repeats (shown below in underlined boldface type) are added to give the $(TTTA)_n$ 4 allele length of 131 bp. Nucleotide position numbers with respect to the Nickerson reference sequence will be off from that point on:

4501 TCTTTTAGTA GCTGTGGGGT TTTGTTGTTG TTCTTCTGTT TTTGCTTAGT ATCTGACTAC
4561 TTTTTAATTA TAAAAAGAGA TGTATCTAAA CAAAATAGAG ATTGTTATCA GAAGTTCACA
4621 ACATTTATTA AAAATTTTTT CACCTGGACA AGAGTCTAAA GCAGCATAAA AATATGGTCT
4681 GCTATATTCT AAACCATCAG TCTTAAGAGA TCTGTGTCTC AGCTTAAGAG AAAATACATT
4741 TAATAGACAG TAACACAAAT AAGAAAAAAA TCTGACCAAG GATAGTGGGA TATAGAAGAA
4801 AAAACATTCC AAGAATTATT TTA**TTTATTTA**TTTATTT ATTTATTTAT TTATTTATTT ATTTATTTAT
4861 TTTTGAGACA CGGTCTCGCT CAGTTACCCA GGCTGGAGTG CAGCGGCGCA ATCTTAACTC
4921 ACTGCAACCT CTGCTTTCCG GTTCAAGCGA TTCTCCTGCC TCAGCCTCCT GAGTAACTGG
4981 GATTACAGGC ACCCGCCACC ACGCCCAACT AATTTCTGTA TTTTTCTTAG TAGAAACAGG
5041 GTTTCACCAT GTTGGCCAAG CTAGTCTCAA ACTCCTGACC TCAGGTGATT CACCCACCAA
5101 GGCCTCCCAA AGTGCTGGGA TTACAGGCAT GAGCCACCAT GCCTGGCCTC CAAAAACTCT //

(SEQ. ID. NO.:93).

A statin drug is any 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, including, but not limited to, lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin.

A human subject, particularly a CABG patient, who has a genetic predisposition for non-responsiveness to statin drug treatment possesses an hereditary inclination, susceptibility, or tendency to develop atherosclerotic stenosis of coronary blood vessels, including of a native coronary artery, or of any coronary artery bypass graft using a saphenous vein or any other vein or artery, in a manner that does not

respond to statin drug treatment. It does not mean that at any time such a person will actually develop stenosis of a coronary blood vessel, or graft worsening (graft lumen narrowing). It merely means that he or she has a greater likelihood of developing stenosis, when statin treatment is given; this is in comparison to the general population of individuals who are not homozygous for a mutation in the 3' end of the *LPL* gene, for example for the *Hind*III 2 allele or $(TTTA)_n$ 4 allele, including those who have atherosclerotic coronary artery disease, who are coronary artery bypass graft patients.

A CABG patient is a human subject who is a candidate for coronary artery bypass graft surgery or one who has undergone a coronary artery bypass graft procedure.

Any human tissue containing nucleic acids can be sampled and collected for the purpose of practicing the methods of the present invention. A most preferred and convenient tissue for collecting is blood. Collecting a tissue sample includes in vitro harvest of cultured human cells derived from a subject's tissue or any means of in vivo sampling directly from a subject, for example, by blood draw, spinal tap, tissue smear or tissue biopsy. Optionally, tissue samples are stored before analysis by well known storage means that will preserve a sample's nucleic acids in an analyzable condition, such as quick freezing, or a controlled freezing regime, in the presence of a cryoprotectant, for example, dimethyl sulfoxide (DMSO), glycerol, or propanediol-sucrose. Tissue samples can also be pooled before or after storage for purposes of amplifying them for analysis.

Amplifying nucleic acids from a tissue sample of a subject to obtain amplification products includes any conventional means of amassing sufficient nucleic acid material for analysis. Most preferably, amplification is by conventional polymerase chain reaction (PCR) methods. Alternatively, amplification of nucleic acids is by in vitro cell culture and harvest of the subject's cultured cells, or by multiple sampling from the subject's tissues in vivo and pooling of multiple tissue samples from a subject. Nucleic acids thus amplified are amplification products if they include a non-coding or untranslated nucleotide sequence from the 3' end of the *LPL* gene, for example, the normal locus of the *Hind*III recognition site in intron 8, or the tetranucleotide $(TTTA)_n$ repeat region of intron 6, of the human *LPL* gene.

In a preferred embodiment of the present method, nucleotide sequencing is used to analyze the amplification products of the nucleic acids in a tissue sample to detect homozygosity for a mutation in the 3' end of human *LPL*. The skilled artisan can detect the mutation by any nucleotide sequencing means, for example conventional dideoxy sequencing or preferably by using a commercially available automated sequencer, then comparing the subject's nucleotide sequences to other known human *LPL* sequences available in genomic sequence databases, such as GenBank.

In a most preferred embodiment that employs nucleotide sequencing, sequencing of 3' end *LPL* sequences is accomplished by using fluorescence-based single strand conformation polymorphism analysis (SSCP), a routine and reliable means of identifying point mutations, small insertions or deletions. (J.S. Ellison, *Fluorescence-based mutation detection. Single-strand conformation polymorphism analysis [F-SSCP], Mol. Biotechnol. 5(1):17-31 [1996];* H. Iwahana *et al., Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, Biotechniques 21(3):510-14, 516-19

[1996]; R. Makino *et al.*, *F-SSCP: fluorescence-based polymerase chain reaction-single-strand conformation polymorphism [PCR-SSCP]*, PCR Methods Appl. 2(1):10-13 [1992]). An automated system may be used, such as an Applied Biosystems DNA sequencer, equipped with GENESCAN 672®, Genotyper®, or another appropriate analytical software package.

Optionally, high throughput analysis may be achieved by PCR multiplexing techniques well known in the art. (E.g., Z. Lin *et al.*, *Multiplex genotype determination at a large number of gene loci*, Proc. Natl. Acad. Sci. USA 93(6):2582-87 [1996]).

In a most preferred embodiment, nucleotide sequencing is unnecessary for analyzing the amplification products. For example, heteroduplex analysis on high resolution gel matrices are employed to detect even single nucleotide polymorphisms. (M.T. Hauser *et al.*, *Generation of co-dominant PCR-based markers by duplex analysis on high resolution gels*, Plant. J. 16(1):117-25 [1998]). The PCR/OLA procedure can be used for analyzing amplification products to detect SNPs in the 3' end of the human *LPL* gene. (B.R. Glick and J. J. Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press, Washington, D.C., pp. 197-200 [1994]). Conformation-sensitive gel electrophoresis of amplification products may also be employed as a means of analysis by the skilled artisan in practicing the methods of the present invention. (A. Markoff *et al.*, *Comparison of conformation-sensitive gel electrophoresis and single strand conformation polymorphism analysis for detection of mutations in the BRCA1 gene using optimized conformation analysis protocols*, Eur. J. Genet. 6(2):145-50 [1998]).

Electrophoresis for analyzing amplification products is done rapidly and with high sensitivity by using any of various methods of conventional slab or capillary electrophoresis, with which the practitioner can optionally choose to employ any facilitating means of nucleic acid fragment detection, including, but not limited to, radionuclides, UV-absorbance or laser-induced fluorescence. (K. Keparnik *et al.*, *Fast detection of a (CA)18 microsatellite repeat in the IgE receptor gene by capillary electrophoresis with laser-induced fluorescence detection*, Electrophoresis 19(2);249-55 [1998]; H. Inoue *et al.*, *Enhanced separation of DNA sequencing products by capillary electrophoresis using a stepwise gradient of electric field strength*, J. Chromatogr. A. 802(1):179-84 [1998]; N.J. Dovichi, *DNA sequencing by capillary electrophoresis*, Electrophoresis 18(12-13):2393-99 [1997]; H. Arakawa *et al.*, *Analysis of single-strand conformation polymorphisms by capillary electrophoresis with laser induced fluorescence detection*, J. Pharm. Biomed. Anal. 15(9-10):1537-44 [1997]; Y. Baba, *Analysis of disease-causing genes and DNA-based drugs by capillary electrophoresis. Towards DNA diagnosis and gene therapy for human diseases*, J. Chromatgr B. Biomed. Appl. 687(2):271-302 [1996]; K.C. Chan *et al.*, *High-speed electrophoretic separation of DNA fragments using a short capillary*, J. Chromatogr B. Biomed. Sci. Appl. 695(1):13-15 [1997]). Any of diverse fluorescent dyes can optionally be used to label primers of the present invention or amplification products for ease of analysis, including but not limited to, SYBR Green I, YIO-PRO-1, thiazole orange, Hex (i.e., 6-carboxy-2',4',7',4,7-hexachlorofluoroscein), pico green, edans, fluorescein, FAM (i.e., 6-carboxyfluorescein), or TET (i.e., 4,7,2',7'-tetrachloro-6-

carboxyfluoroscein). (E.g., J. Skeidsvoll and P.M. Ueland, *Analysis of double-stranded DNA by capillary electrophoresis with laser-induced fluorescence detection using the monomeric dye SYBR green I*, Anal. Biochem. 231(20):359-65 [1995]; H. Iwahana *et al.*, *Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, Biotechniques 21(30:510-14, 516-19 [1996]).

Analyzing the amplification products can also be done by means of restricting the amplification products with one or more restriction enzymes. When the amplification products comprise the normal locus of the *Hind*III recognition site in intron 8 of the human *LPL* gene, the restriction enzyme employed is preferably *Hind*III. Restriction of nucleic acids is followed by separation of the resulting fragments and analysis of fragment length or differential fragment migration in denaturing high-performance liquid chromatography (DHPLC) or gel electrophoresis, as above, including restriction-capillary electrophoresis. For example, this can be achieved by techniques known in the art, such as PCR-restriction fragment-SSCP, which can detect single base substitutions, deletions or insertions. (M. Tawata *et al.*, *A mass screening device of genome by polymerase chain reaction-restriction fragment-single strand conformation polymorphism analysis*, Genet. Anal. 12(3-4):125-27 [1996]; H.H. Lee *et al.*, *Mutational analysis by a combined application of the multiple restriction fragment-single strand conformation polymorphism and the direct linear amplification DNA sequencing protocols*, Anal. Biochem. 205(2);289-93 [1992]).

The present invention also relates to an oligonucleotide primer for detecting a genetic predisposition for non-responsiveness to statin drug treatment in a human. Useful oligonucleotide primers for amplifying the nucleic acids include any 15 to 28-mer nucleotide sequence that hybridizes with a nucleic acid fragment of the Nickerson or Oka reference sequences, under conventional conditions of stringency used for hybridization in PCR, and together in a set with another primer sequence amplifies a non-coding or untranslated region within the 3' end of the human *LPL* gene. A preferred primer is a 20 to 24-mer.

Useful for amplifying non-coding or untranslated nucleic acid sequences from intron 6 ( beginning at position 5988 of the Nickerson reference sequence) through intron 9, is a set of oligonucleotide primers having nucleotide sequences that are fragments of the nucleotide sequences in GenBank accession numbers M76722 (below) and M76723 (opposite strand). The nucleotide sequence of M76722 is the following:

1 GAATTCAAGG TCTGCATTTT CTAGGTATGA ACACTGTGCA TGATGAAGTC TTTCCAAGCC
61 ACACCAGTGG TTCCATGTGT GTGCACTTCC GGTTTGAGTG CTAGTGAGAT ACTTCTGTGG
121 TTCTGAATTG CCTGACTATT TGGGGTTGTG ATATTTTCAT AAAGATTGAT CAACATGTTC
181 GAATTTCCTC CCCAACAGTC TTCCATTACC AAGTAAAGAT TCATTTTTCT GGGACTGAGA
241 GTGAAACCCA TACCAATCAG GCCTTTGAGA TTTCTCTGTA TGGCACCGTG GCCGAGAGTG
301 AGAACATCCC ATTCACTCTG TGAGTAGCAC AGGGGGGCGG TCATCATGGC ACCAGTCCCT
361 CTCCTGCCAT AACCCTTGGT CTGAGCAGCA GAAGCAGAGA GCGATGCCTA GAAAACAAGT
421 CTTTAGTTAA AAAAATCAGA ATTTCAAAAT TGAGGTCTTT CCTCTATTTG ATATTGAGAA
481 AAAAATGCTT CAAATTGGCC ATTTTATTTT CACTTACTAG TTATATTTTT TTATTTATCA
541 TCTTATATCT GTTTATTTCT TTTATAAAGC TGCTGTTAAA CAATATAATT AAACTATCTC
601 AAAAGGTTTG ACATTAAAGA AAATGAGCAA TGGTAACAGG AAACCACTCT ATAGATGTAC
661 ATATAATATG TACAGAAAAT ATAAGTAGTA AGAAGTCCAT GACAAAGTGT TAGCTCTTTT

721 TTTTTTTTTT TTTTTTTTTT TTTTTGAGAT GGAGTCTCTC TCTATTGCCC AGGCTGGAGT
781 GCAGTGATTC GATCTCAGCT CACTGCAACC TCTACCTCCC GAGTTCAAAC AATTCTTCTG
841 TCTCAGCCTC CCGAGTAGCT GGGGCTGCAG GTGCCCACCA CCATGCCCAG CTAATTTTTG
901 TATTTTTAGT AGCGACAGGG TCTCACCATG TTGGCCAAGC TGGTCTTGAA TTCCTGATCT
961 CAGGTGATCC ACCCGCCTCG GCCTCCCAAA GTGCTGGGAT TACAGGTGTG AGCCACCATG
1021 CCCAGCCTAC CCTTTACTAC TAATCAAAGA AATAAAAGTA AGGCAACTTG ATACTTTTAC
1081 AATTACTAGA TGAACAAATC TTTAAAAATA GCCAGTGCAG ACAAGGTGGT GAAGCAGAAC
1141 ATGCGAACCT ACCATGCATC ATTCACGGCT AGAACCCTCC AGGTGCGGAA GGTAGTATTT
1201 TAATAACTTT CCATAGCTAC AAAATATTAT TACATAGAAG GGAGTGATTT TTTTCTAATA
1261 TTTATCCTAA AGAAATAGTC AACAAACATT TTTAAAAACA TCAATTACAG TCGTACCTAT
1321 ACTAGCATAA ATTAGAAACC CAGTATCCAA CATTGAGGCA GTGGGTAAAT GAATCGTGGT
1381 TTATCAAGTC ATTAAAATCA ATCTAGCCTT TAAAAACTAT AATTGTAGGA AACCCAGGAA
1441 AACATAGTAA AAAATGGAAT ATAAAATCTG AAGAGAATAA AGAATAGAGA ATCGTATGTG
1501 TGCTATGATT GTAGCTAAAT AATGTTCAAG TATCAACACA AATTGAAAAG GAATACATGA
1561 AAATGAAAAT TATATTTCTG AATGATTGAC TTCAGGATTT TCTTTTAGAA TTGTATTAAA
1621 TAGTTCATGT CATTAGGATA AATGCTGGAA TGTGGATATA ATTTAAAATA TACTAAATGC
1681 CATCGACCTT CATTTTGAGT TCTTTGTTGG ACATTTTTGT GCATTTTTAA AATATCCCCT
1741 AAATAATAAA GCTATTTATA TTTGGAGAGG AGAAAAAAAA GTGGGGGGCA GGGAGAGCTG
1801 ATCTCTATAA CTAACCAAAT TTATTGCTTT TTTGTTTAGG CCTGAAGTTT CCACAAATAA
1861 GACCTACTCC TTCCTAATTT ACACAGAGGT AGATATTGGA GAACTACTCA TGTTGAAGCT
1921 CAAATGGAAG AGTGATTCAT ACTTTAGCTG GTCAGACTGG TGGAGCAGTC CCGGCTTCGC
1981 CATTCAGAAG ATCAGAGTAA AAGCAGGAGA GACTCAGAAA AAGTAATTAA ATGTATTTTT
2041 CTTCCTTCAC TTTAGACCCC CACCTGATGT CAGGACCTAG GGGCTGTATT TCAGGGGCCT
2101 TCACAATTCA GGGAGAGCTT TAGGAAACCT TGTATTTATT ACTGTATGAT GTAGATTTTC
2161 TTTAGGAGTC TTCTTTTATT TTCTTATTTT TGGGGGGCGG GGGGGGAAGT GACAGTATTT
2221 TTGTATTTCA TGTAAGGAAA ACATAAGCCC TGAATCGCTC ACAGTTATTC AGTGAGAGCT
2281 GGGATTAGAA GTCAGGAATC TCAGCTTCTC ATTTGGCACT GTTTCTTGTA AGTACAAAAT
2341 AGTTAGGGAA CAAACCTCCG AGATGCTACC TGGATAATCA AAGATTCAAA CCAACCTCTT
2401 CCAGAAGGGT GAGATTCCAA GATAATCTCA ACCTGTCTCC GCAGCCCCAC CCATGTGTAC
2461 CCATAAAATG AATTACACAG AGATCGCTAT AGGATTTAAA GCTTTTATAC TAAATGTGCT
2521 GGGATTTTGC AAACTATAGT GTGCTGTTAT TGTTAATTTA AAAAAACTCT AAGTTAGGAT
2581 TGACAAATTA TTTCTCTTTA GTCATTTGCT TGTATCACCA AAGAAGCAAA CAAACAAACA
2641 AAAAAAAAAA GAAAAAGATC TTGGGGATGG AAATGTTATA AAGAATCTTT TTTACACTAG
2701 CAATGTCTAG CTGAAGGCAG ATGCCCTAAT TCCTTAATGC AGATGCTAAG AGATGGCAGA
2761 GTTGATCTTT TATCATCTCT TGGTGAAAGC CCAGTAACAT AAGACTGCTC TAGGCTGTCT
2821 GCATGCCTGT CTATCTAAAT TAACTAGCTT GGTTGCTGAA CACCAGGTTA GGCTCTCAAA
2881 TTACCCTCTG ATTCTGATGT GGCCTGAGTG TGACAGTTAA TTATTGGGAA TATCAAAACA
2941 ATTACCCAGC ATGATCATGT ATTATTTAAA CAGTCCTGAC AGAACTGTAC CTTTGTGAAC
3001 AGTGCTTTTG ATTGTTCTAC ATGGCATATT CACATCCATT TTCTTCCACA GGGTGATCTT
3061 CTGTTCTAGG GAGAAAGTGT CTCATTTGCA GAAAGGAAAG GCACCTGCGG TATTTGTGAA
3121 ATGCCATGAC AAGTCTCTGA ATAAGAAGTC AGGCTGGTGA GCATTCTGGG CTAAAGCTGA
3181 CTGGGCATCC TGAGCTTGCA CCCTAAGGGA GGCAGCTTCA TGCATTCCTC TTCACCCCAT
3241 CACCAGCAGC TTGCCCTGAC TCATGTGATC AAAGCATTCA ATCAGTCTTT CTTAGTCCTT
3301 CTGCATATGT ATCAAATGGG TCTGTTGCTT TATGCAATAC TTCCTCTTTT TTTCTTTCTC
3361 CTCTTGTTTC TCCCAGCCCG GACCTTCAAC CCAGGCACAC ATTTTAGGTT TTATTTTACT

3421 CCTTGAACTA CCCCTGAATC TTCACTTCTC CTTTTTTCTC TACTGCGTCT CTGCTGACTT
3481 TGCAGATGCC ATCTGCAGAG CATGTAACAC AAGTTTAGTA GTTGCCGTTC TGGCTGTGGG
3541 TGCAGCTCTT CCCAGGATGT ATTCAGGGAA GTAAAAAGAT CTCACTGCAT CACCTGCAGC
3601 CACATAGTTC TTGATTCTCC AAGTGCCAGC ATACTCCGGG ACACACAGCC AACAGGGCTG
3661 CCCCAAGCAC CCATTCTCAA AACCCTCAAA GCTGCCAAGC AAACAGAATG AGAGTTATAG
3721 GAAACTGTTC TCTCTTCTAT CTCCAAACAA CTCTGTGCCT CTTTCCTACC TGACCTTTAG
3781 GGCTAATCCA TGTGGCAGCT GTTAGCTGCA TCTTTCCAGA GCGTCAGTAC TGAGAGGACA
3841 CTAAGCATGT GACCTTCACT ACTCCTGTTC TGAATTC (SEQ. ID. NO.:81).

For example, oligonucleotide primer sequences that are useful for amplifying nucleic acids that comprise the normal locus of the *Hind*III recognition site in *LPL* intron 8, include but are not limited to the following sequences (designation after the SEQ. ID. NO. includes the nucleotide position within M76722, e.g., 2701 or 2397, at which the 5'-terminus of the primer sequence begins if it is an upper ["U"; i.e., forward] primer; at which position complementary to a position within M76722 its 3'-terminus ends if it is a lower ["L"; i.e., reverse] primer; and the primer length, e.g., 24 bases):

5'-GCA TCT GCC TTC AGC TAG ACA TTG-3' (SEQ. ID. NO.:1; LPL HindIII:2701L24);
5'-TCT TCC AGA AGG GTG AGA TTC CAA-3' (SEQ. ID. NO.:2; LPL HindIII:2397U24);
5'-GGA AAA CAT AAG CCC TGA ATC-3' (SEQ. ID. NO.:3; LPL HindIII:2236U21);
5'- GAA AAC ATA AGC CCT GAA TCG-3' (SEQ. ID. NO.:4; LPL HindIII:2237U21);
5'-AAC ATA AGC CCT GAA TCG CTC-3' (SEQ. ID. NO.:5; LPL HindIII:2240U21);
5'-CCT GAA TCG CTC ACA GTT ATT-3' (SEQ. ID. NO.6:; LPL HindIII:2249U21);
5'-CTG AAT CGC TCA CAG TTA TTC-3' (SEQ. ID. NO.:7; LPL HindIII:2250U21);
5'-AAT CGC TCA CAG TTA TTC AGT-3' (SEQ ID. NO.:8; LPL HindIII:2253U21);
5'-TTG GCA CTG TTT CTT GTA AGT-3' (SEQ. ID. NO.:9; LPL HindIII:2313U21);
5'-CAC TAT AGT TTG CAA AAT CCC-3' (SEQ. ID. NO.:10; LPL HindIII:2521L21);
5'-CAAACCTCC GAG ATG CTA CCT GGA-3' (SEQ. ID NO.:11; LPL HindIII:2351U24);
5'-AGATGCTACCTG GAT AAT CAA AGA-3' (SEQ. ID. NO.:12; LPL HindIII:2361U24);
5'-GATGCTACC TGG ATA ATC AAA GAT-3' (SEQ. ID. NO.:13; LPL HindIII:2362U24);
5'-CTTCCAGAA GGG TGA GAT TCC AAG-3' (SEQ. ID. NO.:14; LPL HindIII:2398U24);
5'-CCAGAAGGGTGA GAT TCC AAG ATA-3' (SEQ. ID. NO.:15; LPL HindIII:2401U24);
5'-CAGAAGGGTGAG ATT CCA AGA TAA-3' (SEQ. ID. NO.:16, LPL HindIII:2402U24);
5'-CCCACCCAT GTG TAC CCA TAA AAT-3' (SEQ. ID. NO.:17; LPL HindIII:2446U24);
5'-CCACCCATG TGT ACC CAT AAA ATG-3' (SEQ. ID. NO.:18; LPL HindIII:2447U24);
5'-CCCATGTGT ACC CAT AAA ATG AAT-3' (SEQ. ID. NO.:19; LPL HindIII:2450U24);
5'-GTACCCATA AAA TGA ATT ACA CAG-3' (SEQ. ID. NO.:20; LPL HindIII:2457U24);
5'-CCCATAAAATGA ATT ACA CAG AGA-3' (SEQ. ID. NO.:21; LPL HindIII:2460U24);
5'-ATGAATTAC ACA GAG ATC GCT ATA-3' (SEQ. ID. NO.:22; LPL HindIII:2468U24);
5'-ACACAGAGA TCG CTA TAG GAT TTA-3' (SEQ. ID NO.:23; LPL HindIII:2475U24),
5'-TTATAA CAT TTC CAT CCC CAA GAT-3' (SEQ. ID. NO.:24; LPL HindIII:2658L24);
5'-CATCTG CCT TCA GCT AGA CAT TGC-3' (SEQ ID. NO.:25; LPL HindIII:2700L24);
5'-CTGCAT TAA GGA ATT AGG GCA TCT-3' (SEQ. ID. NO.:26; LPL HindIII·2719L24);
5'-AGATCA ACT CTG CCA TCT CTT AGC-3' (SEQ. ID. NO.:27; LPL HindIII:2745L24);

5'-TCT TAT GTT ACT GGG CTT TCA CCA-3' (SEQ. ID NO.:28; LPL HindIII:2781L24);
5'-AGCCTA GAG CAG TCT TAT GTT ACT-3' (SEQ. ID. NO.:29; LPL HindIII:2793L24);
5'-CAGCCT AGA GCA GTC TTA TGT TAC-3' (SEQ. ID. NO.:30; LPL HindIII:2794L24);
5'-ACAGCC TAG AGC AGT CTT ATG TTA-3' (SEQ. ID. NO.:31; LPL HindIII:2795L24);
5'-AGACAGCCT AGA GCA GTC TTA TGT-3' (SEQ. ID. NO.:32; LPL HindIII:2797L24);
5'-CTTTATAACATTTCCATCCCCAAG AT-3' (SEQ. ID. NO.:35; LPL HindIII:2658L26),
5'-TGTACCCATAAAATGAATTACACAGA-3'(SEQ.ID. NO.:36; LPL HindIII:2456U26);
5'-ACCCATAAAATGAATTACACAGAGAT-3'(SEQ.ID. NO..37; LPL HindIII:2459U26);
5'-AAAATGAATTACACAGAGATCGCTAT-3'(SEQ.ID. NO.:38; LPL HindIII:2465U26);
5'-TTACACAGAGATCGCTATAGGATTTA-3' (SEQ.ID. NO.:39; LPL HindIII:2473U26);
5'-CAGCCTAGAGCAGTCTTA TGT TAC T-3' (SEQ. ID. NO.:40; LPL HindIII.2793L25);
5'-ACAGCCTAGAGCAGTCTTATG TTA C-3' (SEQ. ID. NO.:41; LPL HindIII:2794L25);
5'-GACAGCCTAGAGCAGTCTTAT GTT A-3' (SEQ. ID. NO.:42; LPL HindIII:2795L25);
5'-ATAAAATGAATTACACAGAGATCGCTAT-3'(SEQ.ID.NO.:43;LPL HindIII:2463U28);
5'-AAGATTCTTTATAACATTTCCATC CC-3' (SEQ. ID. NO :44; LPL HindIII:2664L26),
5'-AATTACACAGAGATCGCTATAGGATTTA-3'(SEQ.ID.NO.:45;LPL HindIII:2471U28);
5'-ACAGCCTAGAGCAGTCTTATGTTACT-3' (SEQ. ID NO.:46; LPL HindIII:2793L26);
5'-CCC ACC CAT GTG TAC CCA T-3' (SEQ. ID. NO. 47; LPL HindIII:2446U19);
5'-CCA CCC ATG TGT ACC CAT-3' (SEQ. ID. NO.:48; LPL HindIII:2447U18);
5'-CAC CCA TGT GTA CCC ATA AAA-3' (SEQ. ID. NO.:49; LPL HindIII:2448U21);
5'-ACC CAT GTG TAC CCA TAA AA-3' (SEQ. ID. NO.:50; LPL HindIII:2449U20);
5'-GGC TTT CAC CAA GAG ATG ATA A-3' (SEQ. ID. NO.:51; LPL HindIII:2770L22);
5'-GGG CTT TCA CCA AGA GAT GAT A-3' (SEQ. ID NO.:52; LPL HindIII:2771L22);
5'-TGA ATT ACA CAG AGA TCG CTA T-3' (SEQ. ID. NO.:53; LPL HindIII:2469U22);
5'-ACA GAG ATC GCT ATA GGA TTT A-3' (SEQ. ID. NO.:54; LPL HindIII:2477U22);
5'-GTT ACT GGG CTT TCA CC-3' (SEQ. ID. NO.:55; LPL HindIII:2782L17);
5'-CTT ATG TTA CTG GGC TTT CA-3' (SEQ. ID. NO.:56; LPL HindIII:2784L20);
5'-TCT TAT GTT ACT GGG CTT TC-3' (SEQ ID. NO.:57; LPL HindIII:2785L20);
5'-CCA CCC ATG TGT ACC CAT A-3' (SEQ. ID. NO.:58; LPL HindIII:2447U19);
5'-CAC CCA TGT GTA CCC ATA-3' (SEQ. ID. NO.:59; LPL HindIII·2448U18);
5'-ACC CAT GTG TAC CCA TAA-3' (SEQ. ID. NO.:60; LPL HindIII:2449U18);
5'-CCC ATG TGT ACC CAT AAA-3' (SEQ. ID. NO.:61; LPL HindIII:2450U18);
5'-CAA CTC TGC CAT CTC TTA GC-3' (SEQ. ID. NO.:62; LPL HindIII:2745L20);
5'-TCA ACT CTG CCA TCT CTT AG-3' (SEQ. ID. NO.:63; LPL HindIII·2746L20);
5'-ATC AAC TCT GCC ATC TCT TA-3' (SEQ. ID. NO.:64; LPL HindIII.2747L20),
5'-GAA AAC ATA AGC CCT GAA-3' (SEQ. ID. NO.:65; LPL HindIII:2237U18),
5'-AAA ACA TAA GCC CTG AAT C-3' (SEQ. ID. NO.:66; LPL HindIII:2238U19);
5'-ACA TAA GCC CTG AAT CG-3' (SEQ. ID. NO.:67; LPL HindIII:2241U17);
5'-CTG AAT CGC TCA CAG TT-3' (SEQ. ID. NO..68; LPL HindIII:2250U17);
5'-TGA ATC GCT CAC AGT TAT T-3' (SEQ. ID. NO.:69; LPL HindIII:2251U19);
5'-ATC GCT CAC AGT TAT TCA G-3' (SEQ. ID. NO.:70; LPL HindIII:2254U19);

5'-TCG CTC ACA GTT ATT CAG T-3' (SEQ ID. NO.:71; LPL HindIII:2255U19);
5'-CGC TCA CAG TTA TTC AGT G-3' (SEQ. ID. NO.:72, LPL HindIII·2256U19);
5'-AAT CCC AGC ACA TTT AGT AT-3' (SEQ. ID. NO :73; LPL HindIII:2507L20);
5'-ACT ATA GTT TGC AAA ATC CC-3' (SEQ. ID. NO.:74; LPL HindIII·2521L20);
5'-TGA GAG CTG GGA TTA GAA-3' (SEQ. ID. NO.:75; LPL HindIII:2273U18);
5'-GAG AGC TGG GAT TAG AAG T-3' (SEQ. ID. NO.:76; LPL HindIII:2274U19);
5'-AGA GCT GGG ATT AGA AGT C-3' (SEQ. ID. NO.:77; LPL HindIII:2275U19);
5'-AAT CCC AGC ACA TTT AGT AT-3' (SEQ. ID. NO.:78, LPL HindIII:2507L20); and
5'-CCC ACC CAT GTG TAC CCA TA-3' (SEQ. ID. NO.:79; LPL HindIII:2446U20).

Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS: 1-32 or 35-79 can also be used to amplify nucleic acids comprising the normal locus of the *Hind*III recognition site in *LPL* intron 8. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:1-32 and 35-79 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:1-32 and 35-79 and additional nucleotides adjacent to the position(s) based upon the Nickerson reference sequence.

Other primer sequences are useful for amplifying nucleic acid sequences including the $(TTTA)_n$ tetranucleotide repeat region in intron 6. These include SEQ. ID. NOS.:33 and 34, described above and the following primer sequences (designation includes the nucleotide position within the Nickerson reference sequence in Genbank accession AF050163, e.g., 4644 or 4934, at which the 5'-terminus of the primer sequence begins if it is an upper ["U"; i.e., forward] primer; or the position complementary to a position in AF050163 at which its 3'-terminus ends if it is a lower ["L"; i.e., reverse] primer; and primer length, e.g., 24 bases):

5'-CTG GAC AAG AGT CTA AAG CAG CAT-3' ( SEQ. ID. NO.:82; LPL:4644U24);
5'-GAA TCG CTT GAA CCG GAA AG-3' (SEQ. ID. NO.:83; LPL:4934L20:),
5'-ACC ATC AGT CTT AAG AGA TCT GTG-3' (SEQ. ID. NO.:84; LPL:4934L24);
5'-CAC AGA TCT CTT AAG ACT GAT GGT-3' (SEQ. ID. NO.:85; LPL:4693L24);
5'-TTT TTC ACC TGG ACA AGA GT-3' (SEQ. ID. NO.:86; LPL:4636U20);
5'-GGG TAA CTG AGC GAG ACC GT-3' (SEQ. ID. NO.:87; LPL:4870L20);
5'-TTC ACC TGG ACA AGA GTC TA-3' (SEQ. ID. NO..88; LPL:4639U20);
5'-GCT TGA ACC GGA AAG-3' (SEQ ID. NO.:89; LPL:4934L15);
5'-TCA CCT GGA CAA GAG TCT AA-3' (SEQ. ID. NO..90; LPL:4640U20);
5'-CTC CAG CCT GGG TAA CT-3' (SEQ. ID. NO :91, LPL:4882L17); and
5'-ACA AGA GTC TAA AGC AGC AT-3' (SEQ. ID NO.:92; LPL:4648U20).

Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS:33 and 34 or 82-92 can also be used to amplify nucleic acids comprising the $(TTTA)_n$ tetranucleotide repeat region in *LPL* intron 6. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:33-34 and 82-92 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:33-34 and 82-92 and additional nucleotides adjacent to the position(s) based upon the Nickerson reference sequence.

Other primer sequences are useful for amplifying nucleic acid sequences in exon 10 and the 3'-UTR. These include the following primer sequences (SEQ. ID. NOS.:95-106) (designation includes the

nucleotide position within the Oka reference sequence in GenBank accession X52978 X53518, e.g., 2564, at which the 5'-terminus of the primer sequence begins if it is an upper ["U"; i.e., forward] primer; or the position complementary to a position in X52978 X53518 at which its 3'-terminus ends if it is a lower ["L"; i.e., reverse] primer; and primer length, e.g., 22 bases):

5'-ATG AAA AGA GCA TAT GGT GGT T-3' (SEQ. ID. NO.:95; LPL 3' end Oka 2564U22);
5'-TGG CCC AGG TAT ACA TAT GTA ACT A-3' (SEQ. ID. NO.:96; LPL 3' end Oka 2845L25);
5'-GGC CCA GGT ATA CAT ATG TAA CTA A 3' (SEQ. ID. NO.:97; LPL-3' end Oka 2844L25);
5'-TGA AAA GAG CAT ATG GTG GTT C 3' (SEQ. ID. NO.:98; LPL-3' end Oka 2565U22);
5'-GAA AAG AGC ATA TGG TGG TTC-3' (SEQ. ID NO.:99; LPL 3' end Oka 2566U21);
5'-GCC CAG GTA TAC ATA TGT AAC TAA C-3' (SEQ. ID. NO.:100; LPL 3' end Oka 2843L25);
5'-AAA AGA GCA TAT GGT GGT TC-3' (SEQ. ID. NO.:101; LPL 3' end Oka 2567U20);
5'-GGT TCT CTC AGC TCC CAG CCA ACA A-3' (SEQ. ID. NO :102; LPL 3' end Oka 2582U25);
5'-AGC ACA CCA ACA TGG CCC AGG TA-3' (SEQ. ID. NO.:103; LPL 3' end Oka 2869L23);
5'-CTC AGC TCC CAG CCA ACA ACC AGT C-3' (SEQ. ID. NO.:104; LPL 3' end Oka 2588U25);
5'-CAG CAC ACC AAC ATG GCC CAG GTA-3' (SEQ. ID. NO.:105; LPL 3' end Oka 2859L24); and
5'-AGC TCC CAG CCA ACA ACC AGT CTC G-3' (SEQ. ID. NO.:106; LPL 3' end Oka 2591U25).

Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS: 95-106 with respect to its position on the Oka reference sequence can also be used to amplify nucleic acids comprising *LPL* exon 10 and the 3'UTR. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:95-106 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:95-106 and additional nucleotides adjacent to the position(s) based upon the Oka reference sequence.

The present invention also relates to a primer set for detecting a genetic predisposition for non-responsiveness to statin drug treatment in a human. The primer set functions to initiate nucleic acid synthesis in PCR from both the 5' and 3' ends of a nucleic acid template comprising a non-coding or untranslated region of the 3' end of the human *LPL* gene. The primer set comprises any two suitable oligonucleotide primers of the present invention, as described herein, as long as the primer set includes both a forward (upper or "U") and a reverse (lower or "L") primer.

For example, a preferred primer set for amplifying nucleic acids comprising the normal locus of the *Hind*III recognition site in intron 8 of *LPL* includes any pair lower and upper primers from among SEQ. ID. NOS.:1-32 or 35-79 (described above), or primer sequences overlapping any of them with respect to the Nickerson reference sequence. A most preferred set of primers is reverse (lower) primer SEQ. ID. NO.:1 and forward (upper) primer SEQ. ID. NO.:2.

Additional primer sets that are useful for amplifying the region of the $(TTTA)_n$ tetranucleotide repeat include any pair of lower and upper primers from among SEQ. ID. NOS.:33-34 and 82-92 (described above), or primer sequences overlapping any of them with respect to the Nickerson reference sequence. A most preferred embodiment of a primer set for detecting the presence of the $(TTTA)_n$ 4 allele includes primers comprising SEQ. ID. NOS.:33 and 34.

Additional primer sets that are useful for amplifying exon 10 and the 3'UTR include any pair of

lower and upper primers from among SEQ. ID. NOS.:95-106 (described above), or primer sequences overlapping any of them with respect to the Oka reference sequence.

The present invention also relates to a genetic testing kit for detecting in a human subject a genetic predisposition for non-responsiveness to statin drug treatment. The genetic testing kit is a ready assemblage of materials for facilitating the amplifying of nucleic acids from a human subject comprising a nucleotide sequence from a non-coding or untranslated region of the 3' end of the human *LPL* gene and/or analyzing amplification products thereof. A genetic testing kit of the present invention contains at least one oligonucleotide primer of the present invention and preferably comprises a primer set of the present invention, as described above, together with instructions for the practice of the present method. The materials or components assembled in the genetic testing kit are provided to the practitioner stored in any convenient and suitable way that preserves their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

Another preferred embodiment of the genetic testing kit incorporates an array of oligonucleotide primers specific for single nucleotide polymorphisms in the human nucleotide sequence of the 3' end of *LPL*, particularly of non-coding or untranslated regions, preassembled in a "DNA chip" (or "gene chip") configuration for facilitating the amplifying of nucleic acids and the analyzing of amplification products. (E.g., J.G.Hacia *et al.*, *Enhanced high density oligonucleotide array-based sequence analysis using modified nucleoside triphosphates*, Nucleic Acids Res. 26(2):4975-82 [1998]; R.W. Wallace, *DNA on a chip: serving up the genome for diagnostics and research*, Mol. Med. Today 3(9):384-89 [1997]; T. Pastinen *et al.*, *Minisequencing: a specific tool for DNA analysis and diagnostics on oligonucleotide arrays*, Genome Res. 7(6):606-14 [1997]; M.T. Cronin *et al.*, *Cystic fibrosis mutation detection by hybridization to light-generated DNA probe arrays*, Hum. Mutat. 7(3):244-55 [1996]; A.C. Pease *et al.*, *Light-generated oligonucleotide arrays for rapid DNA sequence analysis*, Proc. Natl. Acad. Sci. USA 91(11):5022-26 [1994]; E.M. Southern *et al.*, *Arrays of complementary oligonucleotides for analyzing the hybridisation behaviour of nucleic acids*, Nucleic Acids Res. 22(8):1368-73 [1994]).

The skilled practitioner will appreciate that homozygosity for a mutation in a non-coding or untranslated region of the 3' end of the human LPL gene, such as the *Hind*III 2/2 or $(TTTA)_n$ 4/4 genotypes, is a risk factor for atherosclerotic stenosis in coronary artery disease independent and additive to the use of statin drugs to reduce LDL. For example, the effect of the LPL *Hind*III 2/2 genotype on atherosclerotic graft worsening is of the same magnitude as the use of moderate rather than aggressive drug therapy to lower LDL. Such a genotype apparently does not act via an effect on lipid levels, nor the amount of drug needed to achieve lower levels. However, it is associated with a modest effect on blood pressure.

Using the methods, primers, primer sets, and genetic testing kits of the present invention for detecting a genetic predisposition in a human for non-responsiveness to statin drug treatment for coronary artery disease, the practitioner can identify patients homozygous for a variant allele in a non-coding or

untranslated region of the 3' end of LPL, for example those with the *Hind*III 2/2 or $(TTTA)_n$ 4/4 genotype. These patients are predisposed to develop atherosclerotic progression despite their compliance with aggressive lipid lowering therapy with lovastatin or other statin class drugs.

A high level of LDL-C is an important risk factor of heart disease and atherosclerosis, but it is not the sole risk factor. The present invention provides the practitioner a valuable tool for better characterizing individual patients and identifying those patients likely to need individualized alternative interventions other than LDL-C lowering therapy with statin class drugs. For example, direct blood pressure lowering therapy may be indicated for patients identified as homozygous for a variant genotype in accordance with the present invention, because they tend to have blood pressures at the high end of the normal range. Such treatment can include, for example, angiotensinogen converting enzyme (ACE) inhibitors or $Ca^{2+}$ channel blockers. Alternatively, beta blockers, diuretics, or a combination of modalities can be a more appropriate blood pressure lowering therapy for a given patient. Blood pressure lowering in conjunction with aspirin treatment can prevent heart disease in some patients. (See L. Hansson *et al.*, *Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principle results of the hypertension Optimal Treatment [HOT] randomised trial*, Lancet 351(9118):1755-62 [1998]; *Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischemic heart disease in men at increased risk*, Lancet 351(9098):233-41 [1998]).

For patients identified as homozygous for a variant allele in accordance with the present invention, the practitioner can look at a variety of other known or suspected atherogenic risk factors, beyond LDL-C levels, that may be amenable to treatment in an individual patient. For example, small LDL particle sizes may be amenable to treatment with fibric acid-derivative drugs, e.g., lopid, or high dose niacin. (See J.R. Guyton *et al.*, *Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia*, Am. J. Cardiol. 82(6):737-43 [1998]). High Lp(a) levels may be treatable with niacin, or estrogen replacement therapy in women or testosterone replacement in men.

For some patients identified as homozygous for a variant allele in accordance with the present invention, such as the *Hind*III 2/2 or $(TTTA)_n$ 4/4 genotype, the practitioner can appropriately focus on altering atherogenic life style factors such as diet, smoking, and exercise. (E.g., see J.C. LaRosa, *The role of diet and exercise in the statin era*, Prog. Cardiovasc. Dis. 41(2):137-50 [1998]).

In view of the substantial cost of statin drugs, a secondary benefit to be derived from identifying patients having a genetic predisposition to non-responsiveness to statin drug treatment, for coronary artery disease or high blood pressure, is the cost savings to patients and health care systems that can be gained by relying on more individually suited alternative treatments instead of statin treatment regimens, for those individuals for whom statins are likely to be ineffective. (See D.M. Huse *et al.*, *Cost-effectiveness of statins*, Am. J. Cardiol. 82(11):1357-63 [1998]; P.N. Durrington, *Can we afford to treat hyperlipidaemia as we should? Strategies for rational treatment*, Atherosclerosis 139(Suppl. 1):S1-5 [1998]; J.A. Farmer, *Economic implications of lipid-lowering trials: current considerations in selecting a statin*, Am J.

Cardiol. 82(6A):26M-31M [1998]).

By using the methods, primers, primer sets, and genetic testing kits of the present invention, the practitioner can better individualize the treatment and improve the care of patients with coronary artery disease.

The detailed examples which follow describe the genetic association between variant alleles in non-coding or untranslated regions of the 3' end of the human *LPL* gene and atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment. These examples are intended merely to illustrate and in no way limit the present invention.

## EXAMPLES

Genetic link between mutant *LPL* genotypes and phenotypic atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment.

The following examples describe further data and analyses that support a genetic association between the *LPL HindIII* 2/2 or $(TTTA)_n$ 4/4 genotypes and a phenotype of atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment.

Example 1. Study Design

A genetic association study was conducted by a within-case comparison ancillary to the Post Coronary Artery Bypass Graft Trial. (The Post Coronary Artery Bypass Graft Trial Investigators. *The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in saphenous-vein coronary-artery bypass grafts*, N. Engl. J. Med. 336:153-62 [1997]). A two stage design was followed. First, EBV-transformed lymphoblastoid cell lines were established for subjects from the Los Angeles (L.A.) cohort providing a permanent source of DNA for testing hypotheses related to atherosclerosis-related candidate genes. Then, significant results were tested in a second stage by genotyping all available subjects in the post-CABG trial using DNA isolated from whole blood shipped to Cedars-Sinai Medical Center from the other participating centers.

Participants were randomly assigned, following a two by two design, to receive 1) lovastatin therapy to lower the LDL-cholesterol level to within the range of 93-97 mg/dl (aggressive treatment group) or 132-136 mg/dl (moderate treatment group), and 2) placebo or low-dose warfarin (Post-CABG, 1997). Coronary angiograms of 1351 subjects at enrollment and an average of 4.3 years later were compared using a quantitative assessment of the severity of graft stenosis. Graft worsening was defined as a decrease in lumen diameter of 0.6mm or more. The percentage of subjects with worsening of one or more grafts was 39% in the aggressive treatment group compared to 51% in the moderate treatment group ($p<0.001$) and the mean percentage of grafts per patient showing worsening was 27% in the aggressive treatment group compared to 39% in the moderate treatment group ($p<0.001$).

These results demonstrated the efficacy of lowering LDL-cholesterol levels with statin drug treatment in reducing the risk of graft worsening in most CABG patients. No effect of the warfarin

treatment on graft worsening was observed.

Example 2. Subjects

A total of 1351 subjects from seven clinical centers throughout North America were included in the clinical trial and all were eligible as participants. Genetic material was received from 891 subjects who were included in this ancillary study. Inclusion criteria for the clinical trial were: bypass surgery 1-11 years prior to the study; an LDL-cholesterol level of 130-175 mg/dl; and at least one patent vein graft as determined by angiography. Subjects were excluded if there was: (a) the likelihood of revascularization or death within the study period of 5 years; (b) unstable angina or myocardial infarction within six months before the start of the trial; (c) severe angina; (d) heart failure; or (e) contraindications to the study medications. *Id.* Subjects were randomly assigned in a two by two factorial design for treatment to lower LDL-cholesterol levels aggressively (target LDL 93-97 mg/dl) or moderately (target LDL 132-136 mg/dl) with lovastatin and cholestyramine if needed, and for treatment with either placebo or warfarin sufficient to maintain an international normalized ratio of less than 2. *Id.* Graft worsening was determined by comparing the initial angiogram at enrollment with a follow-up angiogram repeated an average of 4.3 years later. "Worsening" was defined as a reduction in diameter $\geq$0.6mm in diameter. "Subjects with worsening" were defined as those subjects with one or more grafts showing worsening.

Example 3. Data Collection

Questionnaire data regarding demographics, family and medical history, and angiographic and clinical data were collected as part of the post-CABG trial. Additional family history data were collected from 891 subjects in the genetic ancillary study.

Example 4. DNA

During years 2-3 of the clinical trial, cell lines from 224 subjects in the L.A. cohort were established by transformation of peripheral blood lymphocytes with Epstein-Barr virus (EBV). (M. A. Anderson and J.F. Gusella, *Use of cyclosporin-A in establishing Epstein-Barr virus transformed human lymphoblastoid cell lines*. In Vitro 21:856-58 [1984]; S. Pressman and J. I. Rotter, *Epstein-Barr virus transformation of cryopreserved lymphocytes, prolonged experience with technique, letter to the editor*, Am. J. Hum. Genet. 49:467 [1991]). During years 4-5, whole blood was collected from an additional 667 subjects from the other centers. Thus, DNA was available from a total of 891 subjects. DNA was isolated following standard protocols. (B.G. Herrman and A. Frischauf, *Isolation of Genomic DNA, Methods in Enzymology* 152:180-83 [1987]).

Example 5. Genotyping

Conventional agarose gel techniques were used to genotype the LA cohort for the biallelic *LPL Hind*III polymorphism following Heizmann *et al.* (C. Heizmann *et al.*, *RFLP for the human lipoprotein*

*lipase (LPL) gene: HindIII*, Nucleic Acids Res. 15:6763 [1987]). DNA samples from the remaining subjects were genotyped for this polymorphism as well as four additional LPL polymorphisms using fluorescent semi-automated technology. In Figure 1(a), the location of polymorphisms in the LPL gene was assembled from information in GenBank, accession numbers G187209, G34390, M76722, and M76723, and other published sources. (F. Mailly *et al.*, *A common variant in the gene for lipoprotein lipase (asp9-asn): functional implications and prevalence in normal and hyperlipidemic subjects*, Arterioscler. Thromb. Vasc. Biol. 15:468-78 [1995]; P.W.A. Reymer *et al.*, *A lipoprotein lipase mutation (asn291ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis*, Nat Genet 1995;10:28-34 [1995]; C. Heizmann *et al.*, *DNA polymorphism haplotypes of the human lipoprotein lipase gene: possible association with high density lipoprotein levels*, Hum. Genet. 86:578-84 [1991]; G. Zuliani and H.H. Hobbs, *Tetranucleotide repeat polymorphism in the LPL gene*, Nucleic Acids Res. 18:4958 [1990]).

Marker genotypes were determined using a PCR with primers listed below as recommended by the manufacturer of Ampli-Taq Gold (Perkin Elmer, Foster City, CA) in a Perkin Elmer 9600 thermocycler. (All PCR runs began with 95° for 10 min. to activate the polymerase). After digestion with the appropriate restriction enzyme, PCR products for each subject were pooled from all five genotyping reactions and run together on 6% Long Ranger gels in a semi-automated DNA sequencer (ABI 373 DNA sequencer, Applied Biosystems, Foster City, CA) with gel processing using Genescan and Genotyper software.

D9N (exon 2). The assay of Mailley *et al.* (1995) was redesigned using the sequence in GenBank accession G187209 so that the forward primer (5'-Hex-ACT CCG GGA ATG AGG T; SEQ. ID. NO.:107) carried the detection dye and the reverse primer (CCA GAA AGA AGA GAT TTT GTC; SEQ. ID. NO.:108) introduced a *Sal*I restriction site if the PCR fragment carried the mutated D9N allele, and resulted in a 98 bp fragment for the D allele (1 allele) and a 77 bp fragment for the N allele (2 allele) after *Sal*I digestion. PCR conditions were 35 cycles of 94°C 30 sec, 46°C 30 sec, 72°C 30 sec.

N291S (exon 6). The procedure of Reymer *et al.* (1995) was followed with Hex added to the forward primer. PCR conditions were 35 cycles of 94°C 30 sec, 60°C 30 sec, 72°C 30 sec. The reverse primer introduces a partial *Rsa*I site so that the N allele gave a 242 bp fragment (1 allele) and the S allele gave a 218 bp fragment (2 allele) after the *Rsa*I digestion.

*Pvu*II (intron 6). The assay of Li *et al.* (S. Li *et al.*, *PvuII RFLP at the human lipoprotein [LPL] gene locus*, Nucleic Acids Res. 16:2358 [1988]) was redesigned using the sequence in GenBank accession number g34390 so that the resulting fragments would run less than 350 bp in size on the ABI 373. The forward primer was 5'-Tet-CTG CTT TAG ACT CTT GTC CAG GTG (SEQ. ID. NO.:109) and the reverse primer was 5'-GGG TTC AAG GCT CTG TCA GTG TCC (SEQ. ID. NO.:110). PCR conditions

were 35 cycles of 94°C 30 sec, 55°C 30 sec, 72°C 30 sec. A 155 bp fragment was detected if the *Pvu*II site was present (1 allele) and a 282 bp fragment was detected if the *Pvu*II site was absent (2 allele).

(TTTA)$_n$ (intron 6). The procedure of Zuliani and Hobbs (1990) was followed using FAM-labeled GZ-15 primer (5'-CCT GGG TAA CTG AGC GAG ACT GTG TC-3'; SEQ. ID. NO.:33) and GZ-14 primer (5'-ATC TGA CCA AGG ATA GTG GGA TAT A-3'; SEQ. ID. NO.:34). PCR conditions were 35 cycles of 94°C 30 sec, 68°C 3 min. Allele 1 ran at a size of 119 bp, 2 at 123 bp, 3 at 127 bp, 3 at 127 bp, 4 at 131 bp, and 5 at 135 bp.

*Hind*III (intron 8). The assay of Heinzmann *et al.* (1987) was used for stage 1 and then was redesigned for stage 2 using the sequence in GenBank accession numbers M76722 and M76723. Reverse primer was 5'-Fam-GCA TCT GCC TTC AGC TAG ACA TTG (SEQ. ID. NO.:1) and forward primer was 5'-TCT TCC AGA AGG GTG AGA TTC CAA (SEQ. ID. No.:2). PCR conditions were the same as described above for *Pvu*II. Using this primer set of SEQ. ID. NOS.:1 and 2, a 228 bp fragment was detected if the *Hind*III restriction site was present (1 allele) and a 330 bp fragment if absent (2 allele).

Example 6. Statistical Methods

Differences in baseline characteristics between treatment groups and between genotype groups were tested by one-way analysis of variance or Chi-square tests. Log-transformed HDL and TG values were used to perform all statistical analyses in order to adjust for their skewed distributions, but are presented in the tables as untransformed means ± SE. The association between graft worsening and LPL genotype was tested by Chi-square test. Mantel-Haenszel statistics were used for testing the interactions between genotypes and treatment groups. The proportion of grafts showing worsening per subject was used as the quantitative measurement of graft worsening, and multiple regression was performed to for this proportion as a function of genotype and treatment group to identify predictors. Adjusted variables for this trait included age, gender, body mass index, smoking status, number of years since CABG, systolic and diastolic blood pressure, current medicine use, and family history as listed in Table 1. All statistical analyses were carried out with SAS software (version 6.12, SAS Institute, Cary, NC).

Example 7. Baseline Characteristics

Table 1(a) compares the baseline characteristics of the subjects in the aggressive and moderate drug treatment groups. Minor differences were observed in the percent of subjects with a history of stroke, percent using diabetic therapies, systolic and diastolic blood pressures, and baseline LDL levels. The highly significant difference in the steady state levels of total cholesterol and LDL-cholesterol between these two groups reflects the effect of the drug treatment. As shown in Table 1(b), significant differences were observed between the 891 subjects in the genetic study and the 460 subjects who were not included: frequency of prior myocardial infarction, 46% vs. 55%, p=0.001; smoking 9% vs. 14%, p=0.005; mean years from CABG to enrollment, 4.7 vs. 5.2 years, p<0.001; and aspirin use, 79% vs. 69%, p=0.001.

Table 1. Characteristics of Subjects by Treatment Group (1a) or by Inclusion or Exclusion in Genetic Study (1b).

| Characteristic | 1a: By Treatment Group | | | 1b: By Inclusion or Exclusion in Genetic Study | | |
|---|---|---|---|---|---|---|
| | Aggressive (N=430) | Moderate (N=406) | p | Included (N=891) | Excluded (N=460) | p |
| Age (yr, mean(SE) | 62.2±0.4 | 61.5±0.4 | | 62 | 61 | |
| Caucasian (%) | 94 | 96 | | 94 | 96 | |
| Male (%) | 91 | 92 | | 92 | 93 | |
| Body mass index (kg/m$^2$, mean(SE ) | 27.2±0.2 | 27 3±0.2 | | | | |
| Current smoking (%) | 15 | 9 | | 9 | 14 | 0.005 |
| History of myocardial infarction (%) | 45 | 48 | | 46 | 55 | 0.001 |
| Time between CABG and enrollment (yr, mean(SE) | 4.7±0.1 | 4.8±0.1 | | 4.7 | 5.2 | <0 001 |
| Ejection fraction (%): | 56 | 57 | | 57 | 57 | |
| Family history of (%): | | | | | | |
| Coronary artery disease | 76 | 70 | | 70 | 71 | |
| Diabetes | 36 | 33 | | * | | |
| Hypertension | 58 | 56 | | | | |
| Peripheral vascular disease | 19 | 21 | | | | |
| Stroke | 41 | 48 | 0.05 | | | |
| Current medications (%): | | | | | | |
| Aspirin | 82 | 77 | | 79 | 69 | 0.001 |
| Beta-blocker | 25 | 24 | | 24 | 26 | |
| Calcium-channel blocker | 27 | 21 | | 24 | 23 | |
| Insulin or oral antidiabetic agent | 10 | 6 | 0.03 | 10 | 8 | |
| Thiazide diuretic | 12 | 9 | | 11 | 11 | |
| Systolic blood pressure (mmHg, mean(SE) | 134.4±0 8 | 133.0±0.9 | 0 03 | 134.0 | 134.8 | |
| Diastolic blood pressure (mmHg, mean(SE) | 79.3±0.4 | 79.7±0.4 | 0.02 | 79.6 | 80.1 | |
| Baseline lipid levels (mg/dl):† | | | | | | |
| Total cholesterol | 227.3±1.2 | 227.4±1.3 | | 226.9 | 226.4 | |
| LDL cholesterol | 156.1±1.0 | 155.3±1.0 | 0.04 | 155.3 | 155.8 | |
| HDL cholesterol | 39.3±0.5 | 39.5±0.5 | | 39.4 | 39.0 | |
| Triglycerides | 160.3±73.2 | 162.2±3.7 | | 161.1 | 157.6 | |
| Steady state lipid levels (mg/dl)· | | | | | | |
| Total cholesterol | 172.6±1.6 | 209.5±1.6 | 0.001 | 191.1 | 194.3 | |
| LDL cholesterol | 97.1±1.3 | 133.0±1.3 | 0.001 | 120.4 | 114.6 | 0.002 |
| HDL cholesterol | 44.9±0.6 | 43.7±0.6 | | 44.3 | 42.0 | 0.001 |
| Triglycerides | 157.4±4.5 | 164.5±4.4 | | 163.1 | 160.6 | |

Drug treatment groups and included/excluded in genetic study groups were compared by the analysis of variance
Blank p values were nonsignificant.
For the LPL HindIII genotype, "1" indicates the presence, "2" the absence, of the restriction site in intron 8.
Complete data for every category in this table was available for 836 of the 891 subjects in this study.
*Comparable family history data is unavailable on subjects that were not included in the genetic study and so these groups cannot be compared for these characteristics
† Values listed are those measured most recently before enrollment.
To convert cholesterol values to mmol/l, multiply by 0.02586;
to convert triglyceride values to mmol/l, multiply by 0.01129.

Example 8. *LPL Hind*III and Graft Worsening in L.A. Cohort

Genotyping of the L.A. cohort for the *LPL Hind*III polymorphism demonstrated that the proportion of subjects with graft worsening increased with the number of *Hind*III 2 alleles: 42% in those with no *Hind*III 2 allele, 54% in those with one, and 72% in those with two ($X^2$ 2x3 test of association, p=0.05). Further, the percent of grafts showing worsening was calculated per subject and the mean of this percentage also increased with the number of *LPL Hind*III 2 alleles, with 22% in the subjects with *Hind*III 1/1, 31% in subjects with 1/2, and 53% in subjects with 2/2 (analysis of variance, p=0.001).

Example 9. *LPL* HindIII and Graft Worsening in All Subjects

With this result, the remaining 667 subjects were genotyped. A comparison of the percent of subjects with graft worsening for the two *LPL Hind*III genotypes for all 891 subjects is shown in Table 2. A significant difference in the percent of subjects showing graft worsening was observed between those with the *LPL Hind*III 2/2 genotype compared to those with the *LPL Hind*III 1/1 and 1/2 genotypes combined; 58% of those with the *LPL Hind*III 2/2 genotype exhibited worsening compared with 42% of those with either 1/1 or 1/2 (odds ratio=1.9, 95% confidence interval 1.2-3.2, p=0.011). The mean proportion of grafts showing worsening per subject was also significantly increased for those with the LPL HindIII 2/2 genotype (40% for *Hind*III 2/2 compared with 27% for *LPL Hind*III 1/1 and 1/2; p=0.0066). There were no significant differences in graft worsening between subjects with the *LPL Hind*III 1/1 and 1/2 genotypes.

Table 2. Graft Worsening and *LPL HindIII* Genotype.

| Phenotype | LPL HindIII Genotype | | |
|---|---|---|---|
| | 2/2 (N=65) | 1/1 & 1/2 (N=723) | p value |
| Subjects with Worsening (%) | 58 | 41 | 0.011 |
| Mean Grafts with Worsening/Subject (%) | 40 | 27 | 0.0066 |

"Subjects with worsening" defined as subjects with one or more grafts showing worsening, defined as a decrease (0.6 mm in vessel diameter; "mean grafts with worsening/subject" defined as the mean number of grafts showing worsening/total number of grafts per subject. Complete worsening data were available for 788 subjects. "Subjects with worsening" were compared using the Chi square test of association; "mean grafts with worsening/subject" were compared using analysis of variance. For the LPL HindIII genotype, "1" indicates the presence, "2" the absence, of the restriction site in intron 8.

Example 10. *LPL* Genotypes and Graft Worsening

Four additional LPL polymorphisms were tested for association with graft worsening in the entire genetic study cohort (Fig. 1). Complete worsening data were available for 792 subjects; complete genotyping data for each marker represented in Figure 1: D9N (exon 2; Mailley *et al.* [1995], N291S (exon 6; Reymer *et al.* [1995]), *Pvu*II (intron 6; "1"=site is present, "2"=site is absent; S. Li *et al.*[1988]), $(TTTA)_n$ (intron 6; allele 1 is 119 bp, 2 is 123 bp, 3 is 127 bp, 4 is 131 bp, 5 is 135 bp; D.-A. Wu *et al.*, *Quantitative trait locus mapping of human blood pressure to a genetic region at or near the lipoprotein gene locus on chromosome 8p22*, J. Clin. Invest. 97:2111-18 [1996]), *Hind*III (intron 8; "1"= site is present, "2"=site is absent; C. Heizmann *et al.* [1987]). A designation of "X" is an abbreviation for "other" genotypes. The percent of subjects with graft worsening is the percent of subjects with one or more grafts showing a reduction in diameter ≥0.6 mm.

Only the $(TTTA)_n$ and *Hind*III polymorphisms were significantly associated with graft worsening by the Chi square test of association. There was no association between graft worsening and the functional D9N and N291S polymorphisms and also no association with the PvuII polymorphism. In contrast, the 4/4 genotype of the $(TTTA)_n$ polymorphism was associated with graft worsening: 63% of $(TTTA)_n$ 4/4 subjects had worsening of one or more grafts compared to 43% of subjects with other $(TTTA)_n$ genotypes (OR=2.2, 95%CI 1.1-4.6; p=0.027). The $(TTTA)_n$ 4 allele was found to be in strong linkage disequilibrium with the *Hind*III 2 allele (p<0.001, data not shown). Consequently, the combined genotype of $(TTTA)_n$ 4/4 and HindIII 2/2 was also associated with graft worsening at a significance level similar to the $(TTTA)_n$ 4/4 or HindIII 2/2 genotypes alone.

Graft worsening was significantly associated with the *LPL Hind*III 2/2 genotype and tetranucleotide $(TTTA)_n$ 4/4 polymorphisms, both individually and together. The *LPL Hind*III 2/2

polymorphism did not appear to be acting through any lipid variables, but was associated with significant differences in systolic and diastolic blood pressure.

In contrast, no associations between clinical endpoints and the *LPL* D9N, N291S, or *Pvu*II polymorphisms were observed, indicating that the as yet unknown functional mutation associated with graft worsening is in linkage disequilibrium with the $(TTTA)_n$ and *Hind*III polymorphisms, and thus resides in the 3'-end of the *LPL* gene.

Multiple regression analysis demonstrated that there were no differences in the baseline or steady-state serum lipid values, or the response to lipid-lowering therapy between those subjects with the *LPL Hind*III 2/2 genotype and those with the other *Hind*III genotypes (i.e., 1/1 or 1/2). While the present invention is not committed to any particular mechanism, this observation indicates that the LPL polymorphism does not act through an effect on LDL-cholesterol. This result is congruent with that of Peacock *et al.* (1992) who observed an association between the *LPL Hind*III 2 allele and the angiographic severity of atherosclerosis without observing concomitant differences in the mean fasting serum lipid levels in a comparison of young myocardial infarction survivors and age-matched controls.

Some significant differences in important risk factors for atherosclerosis were observed among the group of subjects in the genetic study described herein, including: the frequency of prior myocardial infarction, smoking, aspirin use, and mean years from CABG to enrollment. But if a survival bias occurred, it would lead to an underestimate of the effect of the *LPL Hind*III 2/2 genotype on the risk of graft worsening. Further, in the 891 subjects for which DNA was available, there were no important differences between the aggressive and moderate treatment groups as to the effect of HindIII 2/2 upon responsiveness to statin treatment, as described below.

Example 11. Characteristics of the *Hind*III 2/2 Genotype Group

To investigate potential mechanisms for the association between the LPL *Hind*III 2/2 genotype and graft worsening, baseline characteristics and response of the subjects to the lipid-lowering action of lovastatin were compared between subjects (Table 3). There were no differences observed between the baseline values for total cholesterol, HDL-cholesterol, and triglycerides. However, a small difference in LDL-cholesterol was observed, 159.6±2.1 mg/dl for subjects with *Hind*III 2/2 compared with 155.0±0.7 for 1/1 and 1/2, p=0.04. There were no differences in any of the lipid values attained as a result of drug treatment during the trial, nor was the amount of drug necessary to achieve target lipid values significantly different between the two genotype groups. In contrast to the essentially similar lipid profile of the *LPL Hind*III genotype groups, the *Hind*III 2/2 subjects did vary consistently on one set of physiologic parameters. They had a higher average blood pressure, systolic pressure 138.6±2.1 mmHg vs. 133.7±0.6 for subjects with other genotypes, p=0.03; and diastolic pressure 82.1±1.0 mmHg vs. 79.4±0.3 for subjects with other genotypes, p=0.02.

Multiple regression analysis showed that graft worsening or stenosis was associated with an interaction between the *LPL* genotype and blood pressure. The *LPL Hind*III 2/2 effect on blood

pressure observed here probably has little effect in normal subjects. However, in the presence of ongoing vascular pathology or clinical atherosclerosis, a modest change due to a genetic factor might exert a greater effect. For example, while a blood pressure increase within the normal range has little effect in the general population, slight increases in blood pressure are a significant risk factor for nephropathy in type I diabetes, such that blood-pressure lowering intervention is recommended for some normotensive type I diabetic subjects. (J. Barzilay *et al.*, *Predisposition to hypertension: risk factor for nephropathy and hypertension in IDDM*, Kidney Int. 42:723-30 [1992]; E.J. Lewis *et al.*, *The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy*, N. Engl. J. Med. 329:1456-62 [1993]). Thus, for those patients with an unfavorable *LPL* genotype (e.g., *Hind*III 2/2) other therapies may be indicated in addition to or instead of lipid-lowering statin treatment for prevention of atherosclerotic stenosis.

Table 3. Characteristics of Subjects with HindIII 2/2 Genotype

| Characteristic | LPL HindIII Genotype | |
|---|---|---|
| | 2/2 (N=74) | 1/1 & 1/2 (N=817) |
| Age (yr, mean(SE) | 62.2±0.8 | 61.7±0.3 |
| Caucasian (%) | 97 | 94 |
| Male (%) | 92 | 92 |
| Current smoking (%) | 15 | 9 |
| Body mass index (kg/m$^2$,mean(SE) | 26.7±0.4 | 27.3±0.2 |
| History of myocardial infarction (%) | 47 | 46 |
| Time between CABG and enrollment (yr, mean(SE) | 5.0±0.3 | 4.7±0.1 |
| Ejection fraction (%) | 57 | 57 |
| Family history of (%): | | |
| Coronary heart disease | 76 | 70 |
| Diabetes | 36 | 33 |
| Hypertension | 58 | 56 |
| Peripheral vascular disease | 19 | 21 |
| Stroke | 41 | 45 |
| Current medications (%): | | |
| Aspirin | 82 | 79 |
| Beta-blocker | 23 | 24 |
| Calcium-channel blocker | 26 | 24 |
| Insulin or oral antidiabetic agent | 7 | 8 |
| Thiazide diuretic | 7 | 11 |
| Systolic blood pressure (mmHg, mean(SE)* | 138.6±2.1 | 133.7±0.6 |
| Diastolic blood pressure (mmHg, mean(SE)* | 82.1±1.0 | 79.4±0.3 |
| Baseline lipid levels (mg/dl): | | |
| Total cholesterol | 230.7±2.7 | 226.7±0.9 |
| LDL cholesterol* | 159.6±2.1 | 155.0±0.7 |
| HDL cholesterol | 41.0±1.1 | 39.3±0.3 |
| Triglycerides | 150.8±7.3 | 161.7±2.4 |
| Steady state lipid levels (mg/dl): † | | |
| Total cholesterol | 191.1±4.8 | 191.0±1.3 |
| LDL cholesterol | 115.1±4.3 | 114.7±1.1 |

| | | |
|---|---|---|
| HDL cholesterol | 46.7±1 6 | 44.1±0.4 |
| Triglycerides | 151.0±9.6 | 163.6±3.4 |
| Lipid change (%): | | |
| Total cholesterol | 16.9±2.0 | 15.2±0.6 |
| LDL cholesterol | 53.5±6.9 | 45.1±1.5 |
| HDL cholesterol | 14.1±3.1 | 14.2±1.0 |
| Triglycerides | 6.0±5.8 | 8.2±2.0 |
| Mean lovastatin dose required to reach target lipid levels (mg/day): | | |
| Aggressive treatment group | 37 | 36 |
| Moderate treatment group | 6.0 | 6.5 |

LPL HindIII genotype groups were compared by the analysis of variance.

Blank p values were nonsignificant.

For the LPL HindIII genotype, "1" indicates the presence, "2" the absence, of the restriction site in intron 8.

* p-value less than 0.05.

For the difference between the systolic blood pressures, p=0.03,

for the difference between the diastolic blood pressures, p=0.02,

for the difference between the LDL cholesterol levels, p=0.04.

† Values listed are those measured most recently before enrollment.

To convert cholesterol values to mmol/l, multiply by 0.02586; to convert triglyceride values to mmol/l, multiply by 0.01129.

Example 12. *Hind*III 2/2 Genotype and Statin Drug Treatment

The percent of subjects with graft worsening when stratified by lovastatin treatment group and *LPL Hind*III genotype is shown in Figure 2. Complete *LPL Hind*III genotype data and worsening data were available for 786 subjects.

The highest percentage of subjects with worsening were those with the *Hind*III 2/2 genotype assigned to the moderate lipid-lowering treatment group (65%). The lowest percentage of subjects with worsening were those with the *Hind*III 1/1 or 1/2 genotype assigned to the aggressive lipid-lowering treatment group (35%). Within the *LPL Hind*III 1/1 and 1/2 genotype group, the moderate drug treatment group had a significantly higher percent of subjects with graft worsening than the aggressive treatment group, 49% compared with 35%, odds ratio=1.8, 95% confidence interval 1.3 to 2.4, p<0.001. Within the aggressive treatment group, the *LPL Hind*III 2/2 genotype group had a significantly higher percent of subjects with graft worsening, 54% vs. 35%; OR=2.14, 95%CI 1.11-4.11, p=0.023. The effect of genotype on graft worsening, adjusted for treatment, was significant at p=0.006, OR=2.06, 95%CI 1.23-3.43, and the effect of treatment on graft worsening, adjusted for genotype was significant at p=0.001, OR=1.78, 95%CI 1.32-2.4. The combined effect of both the unfavorable *LPL Hind*III genotype with moderate drug treatment yielded an odds ratio of 3.5 for graft worsening, 95%CI 1.4-8.7, p=0.002.

Using the proportion of grafts with worsening per subject as the dependent variable, the interactions between factors were tested using multiple regression analysis. After adjustments were made for age, sex, body mass index (BMI), smoking, current medication usage, medical history, and family history, the drug treatment group (p=0.0001) and the interaction between the *LPL Hind*III 2/2 genotype and diastolic blood pressure (p=0.0046) remained significant. No interaction between the

dose of lovastatin required to bring each subject to their target LDL-cholesterol level and the *Hind*III 2/2 genotype was observed.

When subjects were stratified by their *LPL Hind*III genotype and drug treatment group, each factor had a similar effect on graft worsening, with odds ratios of 2.1 and 1.8 respectively. The combined effect of both the unfavorable *LPL Hind*III genotype and moderate lipid-lowering yielded an odds ratio for graft worsening of 3.5 (95%CI 1.4-8.7, p=0.002). This analysis demonstrates that the *LPL Hind*III 2/2 genotype is an independent and additive risk factor for worsening of grafts with an odds ratio of the same magnitude as that for lipid-lowering in the post-CABG trial.

The foregoing examples being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.